METHODS AND NUCLEIC ACIDS FOR ANALYSES OF COLORECTAL CELL PROLIFERATIVE DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of United States Patent Application Serial Number 10/603,138, filed 23 June 2003 and entitled METHODS AND NUCLEIC ACIDS FOR ANALYSES OF COLORECTAL CELL PROLIFERATIVE DISORDERS, and which is incorporated by reference herein.

FIELD OF THE INVENTION

The present invention relates to genomic DNA sequences that exhibit altered CpG methylation patterns in disease states relative to normal. Particular embodiments provide methods, nucleic acids, nucleic acid arrays and kits useful for detecting, or for detecting and differentiating between or among colorectal cell proliferative disorders.

SEQUENCE LISTING

A Sequence Listing, pursuant to 37 C.F.R. § 1.52(e)(5), has been provided on compact disc (1 of 1) as a 4.7 MB <u>text</u> file, entitled "SEQ_PROT.doc," and which is incorporated by reference herein in its entirety.

BACKGROUND

The etiology of pathogenic states is known to involve modified methylation patterns of individual genes or of the genome. 5-methylcytosine, in the context of CpG dinucleotide sequences, is the most frequent covalently modified base in the DNA of eukaryotic cells, and plays a role in the regulation of transcription, genetic imprinting, and tumorigenesis. The identification and quantification of 5-methylcytosine sites in a specific specimen, or between or among a plurality of specimens, is thus of considerable interest, not only in research, but particularly for the molecular diagnoses of various diseases.

Correlation of aberrant DNA methylation with cancer. Aberrant DNA methylation within CpG 'islands' is characterized by *hyper-* or *hypomethylation* of CpG dinucleotide

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sequences leading to abrogation or overexpression of a broad spectrum of genes, and is among the earliest and most common alterations found in, and correlated with human malignancies. Additionally, abnormal methylation has been shown to occur in CpG-rich regulatory elements in intronic and coding parts of genes for certain tumors. In colon cancer, for example, aberrant DNA methylation constitutes one of the most prominent alterations and inactivates many tumor suppressor genes such as p14ARF, p16INK4a, THBS1, MINT2, and MINT31 and DNA mismatch repair genes such as hMLH1.

In contrast to the specific hypermethylation of tumor suppressor genes, an overall hypomethylation of DNA can be observed in tumor cells. This decrease in global methylation can be detected early, far before the development of frank tumor formation. A correlation between hypomethylation and increased gene expression has been determined for many oncogenes.

Colorectal Cancer. Colorectal cancer is the fourth leading cause of cancer mortality in men and women, although ranking third in frequency in men and second in women. The 5-year survival rate is 61% over all stages with early detection being a prerequisite for curative therapy of the disease. Up to 95% of all colorectal cancers are adenocarcinomas of varying differentiation grades.

Sporadic colon cancer develops in a multistep process starting with the pathologic transformation of normal colonic epithelium to an adenoma which consecutively progresses to invasive cancer. The progression rate of benign colonic adenomas depends strongly on their histologic appearance: whereas tubular-type adenomas tend to progress to malignant tumors very rarely, villous adenomas, particularly if larger than 2 cm in diameter, have a significant malignant potential.

During progression from benign proliferative lesions to malignant neoplasms several genetic and epigenetic alterations occur. Somatic mutation of the APC gene seems to be one of the earliest events in 75 to 80% of colorectal adenomas and carcinomas. Activation of K-RAS is thought to be a critical step in the progression towards a malignant phenotype. Consecutively, mutations in other oncogenes as well as alterations leading to inactivation of tumor suppressor genes accumulate.

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In the molecular evolution of colorectal cancer, DNA methylation errors have been suggested to play two distinct roles. In normal colonic mucosa cells, methylation errors accumulate as a function of age or as time-dependent events predisposing these cells to neoplastic transformation. For example, hypermethylation of several loci could be shown to be already present in adenomas, particularly in the tubulovillous and villous subtype. At later stages, increased DNA methylation of CpG islands plays an important role in a subset of tumors affected by the so called CpG island methylator phenotype (CIMP). Most CIMP+ tumors, which constitute about 15% of all sporadic colorectal cancers, are characterized by microsatellite instability (MIN) due to hypermethylation of the hMLH1 promoter and other DNA mismatch repair genes. By contrast, CIMP- colon cancers evolve along a more classic genetic instability pathway (CIN), with a high rate of p53 mutations and chromosomal changes.

However, the molecular subtypes do not only show varying frequencies regarding molecular alterations. According to the presence of either micro satellite instability or chromosomal aberrations, colon cancer can be subclassified into two classes, which also exhibit significant clinical differences. Almost all MIN tumors originate in the proximal colon (ascending and transversum), whereas 70% of CIN tumors are located in the distal colon and rectum. This has been attributed to the varying prevalence of different carcinogens in different sections of the colon. Methylating carcinogens, which constitute the prevailing carcinogen in the proximal colon have been suggested to play a role in the pathogenesis of MIN cancers, whereas CIN tumors are thought to be more frequently caused by adduct-forming carcinogens, which occur more frequently in distal parts of the colon and rectum. Moreover, MIN tumors have a better prognosis than do tumors with a CIN phenotype and respond better to adjuvant chemotherapy.

Incidence and mortality rates for this disease increase greatly with age, particularly after the age of 60. Stage of disease at diagnosis also affects overall survival rates. Patients having lesions confined to the colonic wall have a high probability of surviving 5 or more years while patients with metastatic disease have a very low probability of survival. It is thought that most colorectal cancers develop over a course of 5-10 years from a precursor

lesion called an adenomatous polyp. The potential of these lesions to result in adenocarcinoma has been shown to increase with both polyp size and degree of dysplasia. Because of the slow progression of this disease, early detection through routine screening can result in significant improvement of survival rates. Several randomized trials over the last 20 years have shown that screening test can reduce mortality over 30%, even though the tests used were not highly sensitive. The current guidelines for colorectal screening according to the American Cancer Society utilizes one of five different options for screening in average risk individuals 50 years of age or older. These options include 1) fecal occult blood test (FOBT) annually, 2) flexible sigmoidoscopy every five years, 3) annual FPBT plus flexible sigmoidoscopy every five years, 4) double contrast barium enema (DCBE) every five years or 5) colonoscopy every ten years. Even though these testing procedures are well accepted by the medical community, the implementation of widespread screening for colorectal cancer has not been realized. Patient compliance is a major factor for limited use due to the discomfort or inconvenience associated with the procedures. FOBT testing, although a non-invasive procedure, requires dietary and other restrictions 3-5 days prior to testing. Sensitivity levels for this test are also very low for colorectal adenocarcinoma with wide variability depending on the trial. Sensitivity measurements for detection of adenomas is even less since most adenomas do not bleed. In contrast, sensitivity for more invasive procedures such as sigmoidoscopy and colonoscopy are quite high because of direct visualization of the lumen of the colon. No randomized trials have evaluated the efficacy of these techniques, however, using data from case-control studies and data from the National Polyp Study (U.S.) it has been shown that removal of adenomatous polyps results in a 76-90% reduction in CRC incidence. Sigmoidoscopy has the limitation of only visualizing the left side of the colon leaving lesions in the right colon undetected. Both scoping procedures are expensive, require cathartic preparation and have increased risk of morbidity and mortality. Improved tests with increased sensitivity, specificity, ease of use and decreased costs are clearly needed before general widespread screening for colorectal cancer becomes routine.

Molecular disease markers offer several advantages over other types of markers, one advantage being that even samples of very small sizes and/or samples whose tissue

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architecture has not been maintained can be analyzed quite efficiently. Within the last decade a number of genes have been shown to be differentially expressed between normal and colon carcinomas. However, no single or combination of marker has been shown to be sufficient for the diagnosis of colon carcinomas. High-dimensional mRNA based approaches have recently been shown to be able to provide a better means to distinguish between different tumor types and benign and malignant lesions. However its application as a routine diagnostic tool in a clinical environment is impeded by the extreme instability of mRNA, the rapidly occurring expression changes following certain triggers (*e.g.*, sample collection), and, most importantly, the large amount of mRNA needed for analysis (Lipshutz, R. J. et al., Nature Genetics 21:20-24, 1999; Bowtell, D. D. L. Nature genetics suppl. 21:25-32, 1999), which often cannot be obtained from a routine biopsy.

There is a need in the art for a sensitive diagnostic or prognostic assay for colon cell proliferative disorders that is based, at least in part, on detection of differential methylation of CpG dinucleotide sequences, and that has a diagnostic or prognostic accuracy of greater than about 80%, preferably greater than about 85% or about 90%, more preferably greater than about 95%, and most preferably greater than about 98%.

SUMMARY OF THE INVENTION

The present invention provides novel methods for detecting or distinguishing between colorectal cell proliferative disorders. Said method is most preferably utilised for detecting or detecting and distinguishing between one or more of the following: colorectal carcinoma, colon adenoma, inflammatory colon tissue, grade 2 dysplasia colon adenomas less than 1 cm, grade 3 dysplasia colon adenomas larger than 1 cm, normal colon tissue, non-colon normal tissue, body fluids and non-colon cancer tissue. The invention provides a method for the analysis of biological samples for features associated with the development of colon cell proliferative disorders, the method characterised in that at least one nucleic acid, or a fragment thereof, from the group consisting of SEQ ID NO:1 to SEQ ID NO:535 is/are contacted with a reagent or series of reagents capable of distinguishing between methylated and non methylated CpG dinucleotides within the genomic sequence, or sequences of interest.

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The present invention provides a method for ascertaining genetic and/or epigenetic parameters of genomic DNA. The method has utility for the improved diagnosis, treatment and monitoring of colon cell proliferative disorders, more specifically by enabling the improved identification of and differentiation between subclasses of said disorder and the genetic predisposition to said disorders. The invention presents improvements over the state of the art in that it enables a highly specific classification of colon cell proliferative disorders, thereby allowing for improved and informed treatment of patients.

Preferably, the source of the test sample is selected from the group consisting of cells or cell lines, histological slides, biopsies, paraffin-embedded tissue, bodily fluids, ejaculate, urine, blood, and combinations thereof. Preferably, the source is biopsies, bodily fluids, ejaculate, urine, or blood.

Specifically, the present invention provides a method for detecting colon cell proliferative disorders, comprising: obtaining a biological sample comprising genomic nucleic acid(s); contacting the nucleic acid(s), or a fragment thereof, with one reagent or a plurality of reagents sufficient for distinguishing between methylated and non methylated CpG dinucleotide sequences within a target sequence of the subject nucleic acid, wherein the target sequence comprises, or hybridizes under stringent conditions to, a sequence comprising at least 16 contiguous nucleotides of SEQ ID NO:1 to 535, said contiguous nucleotides comprising at least one CpG dinucleotide sequence; and determining, based at least in part on said distinguishing, the methylation state of at least one target CpG dinucleotide sequence, or an average, or a value reflecting an average methylation state of a plurality of target CpG dinucleotide sequences. Preferably, distinguishing between methylated and non methylated CpG dinucleotide sequences within the target sequence comprises methylation state-dependent conversion or non-conversion of at least one such CpG dinucleotide sequence to the corresponding converted or non-converted dinucleotide sequence within a sequence selected from the group consisting of SEQ ID NO:304 to SEQ ID NO:535, and contiguous regions thereof corresponding to the target sequence.

Additional embodiments provide a method for the detection of colon cell proliferative disorders, comprising: obtaining a biological sample having subject genomic DNA; extracting

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the genomic DNA; treating the genomic DNA, or a fragment thereof, with one or more reagents to convert 5-position unmethylated cytosine bases to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridization properties; contacting the treated genomic DNA, or the treated fragment thereof, with an amplification enzyme and at least two primers comprising, in each case a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting SEQ ID NO:304 to SEQ ID NO:535, and complements thereof, wherein the treated DNA or the fragment thereof is either amplified to produce an amplificate, or is not amplified; and determining, based on a presence or absence of, or on a property of said amplificate, the methylation state of at least one CpG dinucleotide sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:58, or an average, or a value reflecting an average methylation state of a plurality of CpG dinucleotide sequences thereof. Preferably, at least one such hybridizing nucleic acid molecule or peptide nucleic acid molecule is bound to a solid phase. Preferably, determining comprises use of at least two methods selected from the group consisting of: hybridizing at least one nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NO:304 to SEQ ID NO:535, and complements thereof; hybridizing at least one nucleic acid molecule, bound to a solid phase, comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NO:304 to SEQ ID NO:535, and complements thereof; hybridizing at least one nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NO:304 to SEQ ID NO:535, and complements thereof, and extending at least one such hybridized nucleic acid molecule by at least one nucleotide base; and sequencing of the amplificate.

Further embodiments provide a method for the analysis of colon cell proliferative disorders, comprising: obtaining a biological sample having subject genomic DNA; extracting

the genomic DNA; contacting the genomic DNA, or a fragment thereof, comprising one or more sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:58 or a sequence that hybridizes under stringent conditions thereto, with one or more methylation-sensitive restriction enzymes, wherein the genomic DNA is either digested thereby to produce digestion fragments, or is not digested thereby; and determining, based on a presence or absence of, or on property of at least one such fragment, the methylation state of at least one CpG dinucleotide sequence of one or more sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:58, or an average, or a value reflecting an average methylation state of a plurality of CpG dinucleotide sequences thereof. Preferably, the digested or undigested genomic DNA is amplified prior to said determining.

Additional embodiments provide novel genomic and chemically modified nucleic acid sequences, as well as oligonucleotides and/or PNA-oligomers for analysis of cytosine methylation patterns within sequences from the group consisting of SEQ ID NO:1 to SEQ ID NO:58.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1, 5, 9, 13, 16, 20, 24, 28 and 32 show ranked matrices of data obtained according to EXAMPLES 1 and 2, and according to CpG methylation differences between the two classes of tissues, using a suitable algorithm. The figures are shown in greyscale, wherein the most significant CpG positions are at the bottom of the matrix with significance decreasing towards the top. Black indicates total methylation at a given CpG position, white represents no methylation at the particular position, with degrees of methylation represented in grey, from light (low proportion of methylation) to dark (high proportion of methylation). Each row represents one specific CpG position within a gene and each column shows the methylation profile for the different CpGs for one sample. The p-values for the individual CpG positions are shown on the right side. The p-values are the probabilities that the observed distribution occurred by chance in the data set.

Figures 2, 6, 10, 17, 21, 25, 29 and 33 show the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figures 3, 7, 11, 14, 18, 22, 26, 30 and 34 show ranked matrices of data, obtained according to EXAMPLES 1 and 2, of the accuracy of the genewise linear support vector machine cross validations between the two classes of tissues, for the best performing markers. The figures are shown in greyscale, wherein the most significant CpG positions are at the bottom of the matrix with significance decreasing towards the top. Black indicates total methylation at a given CpG position, white represents no methylation at the particular position, with degrees of methylation represented in grey, from light (low proportion of methylation) to dark (high proportion of methylation). Each row represents one specific CpG position within a gene and each column shows the methylation profile for the different CpGs for one sample. Accuracy values for each individual genomic region of interest are shown on the right side.

Figures 4, 8, 12, 15, 18, 23, 27, 31 and 35 show the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification. The accuracy of each genomic region is represented as black squares, the specificity as unfilled diamonds, and the sensitivity as unfilled squares. The accuracy as measured on the X-axis shows the fraction of correctly classified samples.

Figure 36 shows the level of methylation determined by different MSP MethyLight assays and HeavyMethyl MethyLight assays. The Y-axis shows the degree of methylation. Tumor samples are represented by white points, and normal colon tissue samples by black points. A significantly higher degree of methylation was observed in tumor samples than in healthy tissue samples.

Figure 37 shows the Receiver Operating Characteristic curve (ROC curve) of the SEQ ID NO:35 -MSP-Methyl-Light-Assay for adenocarcinomas according to EXAMPLE 2. The AUC for the MSP-Methyl-Light-Assay is: 0.94.

Figure 38 shows the Receiver Operating Characteristic curve (ROC curve) of the SEQ ID NO:35 -HM-Methyl-Light-Assay for Adenocarcinoma according to EXAMPLE 3. The AUC for the HM-Methyl-Light-Assay is: 0.91.

Figure 39 shows the level of methylation determined by a SEQ ID NO:35 - HeavyMethyl MethyLight™ assay according to EXAMPLE 3, testing an additional set of colon samples (25 adenocarcinoma, 33 normals, and 13 adenomas). The Y-axis shows the degree of methylation within the region of the SEQ ID NO:35 gene investigated. Adenocarcinoma samples are represented by white squares, and normal colon tissue samples by black diamonds. A significantly higher degree of methylation was observed in tumor samples than in healthy tissue samples. The level of significance as measured using a t-test was 0.00424.

Figure 40 shows the Receiver Operating Characteristic curve (ROC curve) of the SEQ ID NO:35 -HM-Methyl-Light-Assay for Adenocarcinoma and Adenoma according to EXAMPLE 3 (additional sets of samples). The area under an ROC curve (AUC) is a measure for the accuracy of a diagnostic test. The AUC for the HM-Methyl-Light-Assay is 0.81.

Figure 41 shows the Receiver Operating Characteristic curve (ROC curve) of the SEQ ID NO:35 -HM-Methyl-Light-Assay for Adenocarcinoma only according to EXAMPLE 2 (additional sets of samples). The area under an ROC curve (AUC) is a measure for the accuracy of a diagnostic test. The AUC for the HM-Methyl-Light-Assay is: 0.844.

Figure 42 shows the Receiver Operating Characteristic curve (ROC curve) of the SEQ ID NO:35 -HM-Methyl-Light-Assay for Adenenomas according to EXAMPLE 3 (additional sets of samples). The area under an ROC curve (AUC) is a measure for the accuracy of a diagnostic test. The AUC for the HM-Methyl-Light-Assay is: 0.748.

Figure 43 shows the level of methylation in different tumor and healthy tissues determined by a SEQ ID NO 35 -HeavyMethyl MethyLight™ assay according to example 4. The Y-axis shows the degree of methylation within the region of the SEQ ID NO:35 gene investigated. Besides the colon cancer samples only one of the two breast cancer tissues were methylated.

Figure 44 shows the level of methylation in different breast cancer tissues determined by a SEQ ID NO:35 -HeavyMethyl MethyLight™ assay according to EXAMPLE 4. Only one was methylated.

Figure 45 shows the level of methylation in serum samples determined by a SEQ ID NO:35 -HeavyMethyl MethyLight ™ assay according to EXAMPLE 4. The Y-axis shows the degree of methylation within the region of the SEQ ID NO:35 gene investigated.

Figure 46 shows the ROC curve of the SEQ ID NO:34 -MSP-Methyl-Light ™-Assay according to EXAMPLE 9. The AUC is: 0.84.

Figure 47 shows the ROC curve of the SEQ ID NO:29 -MSP-Methyl-Light ™-Assay according to EXAMPLE 10. The AUC is: 0.80.

Figure 48 shows the regression plot of the percentage methylation within SEQ ID NO:35 calculated in each sample using the MSP and HeavyMethyl ™ variants of the MethyLight ™ assay.

Figure 49 shows the ROC curve of the SEQ ID NO:29 -MSP-Methyl-Light ™-Assay according to EXAMPLE 8 (first sample set). The AUC is: 0.93.

Figure 50 shows the ROC curve of the SEQ ID NO:29 -MSP-Methyl-Light ™-Assay according to EXAMPLE 8 (second sample set). The AUC is: 1.

Figure 51 shows the ROC curve of the SEQ ID NO:39 -MSP-Methyl-Light ™-Assay according to EXAMPLE 9. The AUC is: 0.94.

DETAILED DESCRIPTION OF THE INVENTION

Definitions:

The term "Observed/Expected Ratio" ("O/E Ratio") refers to the frequency of CpG dinucleotides within a particular DNA sequence, and corresponds to the [number of CpG sites / (number of C bases × number of G bases)] × band length for each fragment.

The term "CpG island" refers to a contiguous region of genomic DNA that satisfies the criteria of (1) having a frequency of CpG dinucleotides corresponding to an "Observed/Expected Ratio" >0.6, and (2) having a "GC Content" >0.5. CpG islands are typically, but not always, between about 0.2 to about 1 kb, or to about 2kb in length.

The term "methylation state" or "methylation status" refers to the presence or absence of 5-methylcytosine ("5-mCyt") at one or a plurality of CpG dinucleotides within a DNA sequence. Methylation states at one or more particular palindromic CpG methylation sites (each having two CpG CpG dinucleotide sequences) within a DNA sequence include "unmethylated," "fully-methylated" and "hemi-methylated."

The term "hemi-methylation" or "hemimethylation" refers to the methylation state of a palindromic CpG methylation site, where only a single cytosine in one of the two CpG dinucleotide sequences of the palindromic CpG methylation site is methylated (*e.g.*, 5'-CC^{M}GG-3' (top strand): 3'-GGCC-5' (bottom strand)).

The term "hypermethylation" refers to the average methylation state corresponding to an *increased* presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample.

The term "hypomethylation" refers to the average methylation state corresponding to a *decreased* presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample.

The term "microarray" refers broadly to both "DNA microarrays," and 'DNA chip(s),' as recognized in the art, encompasses all art-recognized solid supports, and encompasses all methods for affixing nucleic acid molecules thereto or synthesis of nucleic acids thereon.

"Genetic parameters" are mutations and polymorphisms of genes and sequences further required for their regulation. To be designated as mutations are, in particular, insertions, deletions, point mutations, inversions and polymorphisms and, particularly preferred, SNPs (single nucleotide polymorphisms).

"Epigenetic parameters" are, in particular, cytosine methylations. Further epigenetic parameters include, for example, the acetylation of histones which, however, cannot be directly analyzed using the described method but which, in turn, correlate with the DNA methylation.

The term "bisulfite reagent" refers to a reagent comprising bisulfite, disulfite, hydrogen sulfite or combinations thereof, useful as disclosed herein to distinguish between methylated and unmethylated CpG dinucleotide sequences.

The term "Methylation assay" refers to any assay for determining the methylation state of one or more CpG dinucleotide sequences within a sequence of DNA.

The term "MS.AP-PCR" (Methylation-Sensitive Arbitrarily-Primed Polymerase Chain Reaction) refers to the art-recognized technology that allows for a global scan of the genome using CG-rich primers to focus on the regions most likely to contain CpG dinucleotides, and described by Gonzalgo et al., *Cancer Research* 57:594-599, 1997.

The term "MethyLight™" refers to the art-recognized fluorescence-based real-time PCR technique described by Eads et al., *Cancer Res.* 59:2302-2306, 1999.

The term "HeavyMethyl™" assay, in the embodiment thereof implemented herein, refers to an assay, wherein methylation specific *blocking* probes (also referred to herein as *blockers*) covering CpG positions between, or covered by the amplification primers enable methylation-specific selective amplification of a nucleic acid sample.

The term "Ms-SNuPE" (Methylation-sensitive Single Nucleotide Primer Extension) refers to the art-recognized assay described by Gonzalgo & Jones, *Nucleic Acids Res.* 25:2529-2531, 1997.

The term "MSP" (Methylation-specific PCR) refers to the art-recognized methylation assay described by Herman et al. *Proc. Natl. Acad. Sci. USA* 93:9821-9826, 1996, and by US Patent No. 5,786,146.

The term "COBRA" (Combined Bisulfite Restriction Analysis) refers to the art-recognized methylation assay described by Xiong & Laird, *Nucleic Acids Res.* 25:2532-2534, 1997.

The term "MCA" (Methylated CpG Island Amplification) refers to the methylation assay described by Toyota et al., *Cancer Res.* 59:2307-12, 1999, and in WO 00/26401A1.

The term "hybridization" is to be understood as a bond of an oligonucleotide to a complementary sequence along the lines of the Watson-Crick base pairings in the sample DNA, forming a duplex structure.

"Stringent hybridization conditions," as defined herein, involve hybridizing at 68°C in 5x SSC/5x Denhardt's solution/1.0% SDS, and washing in 0.2x SSC/0.1% SDS at room temperature, or involve the art-recognized equivalent thereof (e.g., conditions in which a hybridization is carried out at 60°C in 2.5 x SSC buffer, followed by several washing steps at 37°C in a low buffer concentration, and remains stable). Moderately stringent conditions, as defined herein, involve including washing in 3x SSC at 42°C, or the art-recognized equivalent thereof. The parameters of salt concentration and temperature can be varied to achieve the optimal level of identity between the probe and the target nucleic acid. Guidance regarding such conditions is available in the art, for example, by Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, N.Y.; and Ausubel et al. (eds.), 1995, Current Protocols in Molecular Biology, (John Wiley & Sons, N.Y.) at Unit 2.10.

The terms "array SEQ ID NO," "composite array SEQ ID NO," or "composite array sequence" refer to a sequence, hypothetical or otherwise, consisting of a head-to-tail (5' to 3') linear composite of all individual contiguous sequences of a subject array (e.g., a head-to-tail composite of SEQ ID NOS:1-71, in that order).

The terms "array SEQ ID NO node," "composite array SEQ ID NO node," or "composite array sequence node" refer to a *junction* between any two individual contiguous sequences of the "array SEQ ID NO," the "composite array SEQ ID NO," or the "composite array sequence."

In reference to composite array sequences, the phrase "contiguous nucleotides" refers to a contiguous sequence region of any individual contiguous sequence of the composite array, but does not include a region of the composite array sequence that includes a "node," as defined herein above.

Overview:

The present invention provides for molecular genetic markers that have novel utility for the analysis of methylation patterns associated with the development of colon cell proliferative disorders. Said markers may be used for detecting or distinguishing between

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colon cell proliferative disorders, thereby providing improved means for the classification and treatment of said disorders.

Bisulfite modification of DNA is an art-recognized tool used to assess CpG methylation status. 5-methylcytosine is the most frequent covalent base modification in the DNA of eukaryotic cells. It plays a role, for example, in the regulation of the transcription, in genetic imprinting, and in tumorigenesis. Therefore, the identification of 5-methylcytosine as a component of genetic information is of considerable interest. However, 5-methylcytosine positions cannot be identified by sequencing, because 5-methylcytosine has the same base pairing behavior as cytosine. Moreover, the epigenetic information carried by 5-methylcytosine is completely lost during, *e.g.*, PCR amplification.

The most frequently used method for analyzing DNA for the presence of 5-methylcytosine is based upon the specific reaction of bisulfite with cytosine whereby, upon subsequent alkaline hydrolysis, cytosine is converted to uracil which corresponds to thymine in its base pairing behavior. Significantly, however, 5-methylcytosine remains unmodified under these conditions. Consequently, the original DNA is *converted* in such a manner that methylcytosine, which originally could not be distinguished from cytosine by its hybridization behavior, can now be detected as the only remaining cytosine using standard, art-recognized molecular biological techniques, for example, by amplification and hybridization, or by sequencing. All of these techniques are based on differential base pairing properties, which can now be fully exploited.

The prior art, in terms of sensitivity, is defined by a method comprising enclosing the DNA to be analyzed in an agarose matrix, thereby preventing the diffusion and renaturation of the DNA (bisulfite only reacts with single-stranded DNA), and replacing all precipitation and purification steps with fast dialysis (Olek A, et al., A modified and improved method for bisulfite based cytosine methylation analysis, *Nucleic Acids Res.* 24:5064-6, 1996). It is thus possible to analyze individual cells for methylation status, illustrating the utility and sensitivity of the method. An overview of art-recognized methods for detecting 5-methylcytosine is provided by Rein, T., et al., *Nucleic Acids Res.*, 26:2255, 1998.

The bisulfite technique, barring few exceptions (*e.g.*, Zeschnigk M, et al., *Eur J Hum Genet.* 5:94-98, 1997), is currently only used in research. In all instances, short, specific fragments of a known gene are amplified subsequent to a bisulfite treatment, and either completely sequenced (Olek & Walter, *Nat Genet.* 1997 17:275-6, 1997), subjected to one or more primer extension reactions (Gonzalgo & Jones, *Nucleic Acids Res.*, 25:2529-31, 1997; WO 95/00669; U.S. Patent No. 6,251,594) to analyze individual cytosine positions, or treated by enzymatic digestion (Xiong & Laird, *Nucleic Acids Res.*, 25:2532-4, 1997). Detection by hybridization has also been described in the art (Olek et al., WO 99/28498). Additionally, use of the bisulfite technique for methylation detection with respect to individual genes has been described (Grigg & Clark, *Bioessays*, 16:431-6, 1994; Zeschnigk M, et al., *Hum Mol Genet.*, 6:387-95, 1997; Feil R, et al., *Nucleic Acids Res.*, 22:695-, 1994; Martin V, et al., *Gene*, 157:261-4, 1995; WO 9746705 and WO 9515373).

The present invention provides for the use of the bisulfite technique , in combination with one or more methylation assays, for determination of the methylation status of CpG dinuclotide sequences within sequences from the group consisting of SEQ ID NO:1 to SEQ ID NO:58. According to the present invention, determination of the methylation status of CpG dinuclotide sequences within sequences from the group consisting of SEQ ID NO:1 to SEQ ID NO:58 has diagnostic and prognostic utility.

Methylation Assay Procedures. Various methylation assay procedures are known in the art, and can be used in conjunction with the present invention. These assays allow for determination of the methylation state of one or a plurality of CpG dinucleotides (*e.g.*, CpG islands) within a DNA sequence. Such assays involve, among other techniques, DNA sequencing of bisulfite-treated DNA, PCR (for sequence-specific amplification), Southern blot analysis, and use of methylation-sensitive restriction enzymes.

For example, genomic sequencing has been simplified for analysis of DNA methylation patterns and 5-methylcytosine distribution by using bisulfite treatment (Frommer et al., *Proc. Natl. Acad. Sci. USA* 89:1827-1831, 1992). Additionally, restriction enzyme digestion of PCR products amplified from bisulfite-converted DNA is used, *e.g.*, the method

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described by Sadri & Hornsby (*Nucl. Acids Res.* 24:5058-5059, 1996), or COBRA (Combined Bisulfite Restriction Analysis) (Xiong & Laird, *Nucleic Acids Res.* 25:2532-2534, 1997).

COBRA. COBRA analysis is a quantitative methylation assay useful for determining DNA methylation levels at specific gene loci in small amounts of genomic DNA (Xiong & Laird, *Nucleic Acids Res.* 25:2532-2534, 1997). Briefly, restriction enzyme digestion is used to reveal methylation-dependent sequence differences in PCR products of sodium bisulfite-treated DNA. Methylation-dependent sequence differences are first introduced into the genomic DNA by standard bisulfite treatment according to the procedure described by Frommer et al. (*Proc. Natl. Acad. Sci. USA* 89:1827-1831, 1992). PCR amplification of the bisulfite converted DNA is then performed using primers specific for the interested CpG islands, followed by restriction endonuclease digestion, gel electrophoresis, and detection using specific, labeled hybridization probes. Methylation levels in the original DNA sample are represented by the relative amounts of digested and undigested PCR product in a linearly quantitative fashion across a wide spectrum of DNA methylation levels. In addition, this technique can be reliably applied to DNA obtained from microdissected paraffin-embedded tissue samples. Typical reagents (*e.g.*, as might be found in a typical COBRA-based kit) for COBRA analysis may include, but are not limited to: PCR primers for specific gene (or methylation-altered DNA sequence or CpG island); restriction enzyme and appropriate buffer; gene-hybridization oligo; control hybridization oligo; kinase labeling kit for oligo probe; and radioactive nucleotides. Additionally, bisulfite conversion reagents may include: DNA denaturation buffer; sulfonation buffer; DNA recovery reagents or kits (*e.g.*, precipitation, ultrafiltration, affinity column); desulfonation buffer; and DNA recovery components.

Preferably, assays such as "MethyLight ™" (a fluorescence-based real-time PCR technique) (Eads et al., *Cancer Res.* 59:2302-2306, 1999), Ms-SNuPE (Methylation-sensitive Single Nucleotide Primer Extension) reactions (Gonzalgo & Jones, *Nucleic Acids Res.* 25:2529-2531, 1997), methylation-specific PCR ("MSP"; Herman et al., *Proc. Natl. Acad. Sci. USA* 93:9821-9826, 1996; US Patent No. 5,786,146), and methylated CpG island amplification ("MCA"; Toyota et al., *Cancer Res.* 59:2307-12, 1999) are used alone or in combination with other of these methods.

MethyLight ™. The MethyLight ™ assay is a high-throughput quantitative methylation assay that utilizes fluorescence-based real-time PCR (TaqMan ™) technology that requires no further manipulations after the PCR step (Eads et al., *Cancer Res.* 59:2302-2306, 1999). Briefly, the MethyLight ™ process begins with a mixed sample of genomic DNA that is converted, in a sodium bisulfite reaction, to a mixed pool of methylation-dependent sequence differences according to standard procedures (the bisulfite process converts unmethylated cytosine residues to uracil). Fluorescence-based PCR is then performed either in an "unbiased" (with primers that do not overlap known CpG methylation sites) PCR reaction, or in a "biased" (with PCR primers that overlap known CpG dinucleotides) reaction. Sequence discrimination can occur either at the level of the amplification process or at the level of the fluorescence detection process, or both.

The MethyLight ™ assay may be used as a quantitative test for methylation patterns in the genomic DNA sample, wherein sequence discrimination occurs at the level of probe hybridization. In this quantitative version, the PCR reaction provides for unbiased amplification in the presence of a fluorescent probe that overlaps a particular putative methylation site. An unbiased control for the amount of input DNA is provided by a reaction in which neither the primers, nor the probe overlie any CpG dinucleotides. Alternatively, a qualitative test for genomic methylation is achieved by probing of the biased PCR pool with either control oligonucleotides that do not "cover" known methylation sites (a fluorescence-based version of the "MSP" technique), or with oligonucleotides covering potential methylation sites.

The MethyLight ™ process can by used with a "TaqMan®" probe in the amplification process. For example, double-stranded genomic DNA is treated with sodium bisulfite and subjected to one of two sets of PCR reactions using TaqMan® probes; *e.g.*, with either biased primers and TaqMan® probe, or unbiased primers and TaqMan® probe. The TaqMan® probe is dual-labeled with fluorescent "reporter" and "quencher" molecules, and is designed to be specific for a relatively high GC content region so that it melts out at about 10°C higher temperature in the PCR cycle than the forward or reverse primers. This allows the TaqMan® probe to remain fully hybridized during the PCR annealing/extension step. As the Taq

polymerase enzymatically synthesizes a new strand during PCR, it will eventually reach the annealed TaqMan® probe. The Taq polymerase 5' to 3' endonuclease activity will then displace the TaqMan® probe by digesting it to release the fluorescent reporter molecule for quantitative detection of its now unquenched signal using a real-time fluorescent detection system.

Typical reagents (*e.g.*, as might be found in a typical MethyLight ™-based kit) for MethyLight ™ analysis may include, but are not limited to: PCR primers for specific gene (or methylation-altered DNA sequence or CpG island); TaqMan® probes; optimized PCR buffers and deoxynucleotides; and Taq polymerase.

Ms-SNuPE. The Ms-SNuPE technique is a quantitative method for assessing methylation differences at specific CpG sites based on bisulfite treatment of DNA, followed by single-nucleotide primer extension (Gonzalgo & Jones, *Nucleic Acids Res*. 25:2529-2531, 1997). Briefly, genomic DNA is reacted with sodium bisulfite to convert unmethylated cytosine to uracil while leaving 5-methylcytosine unchanged. Amplification of the desired target sequence is then performed using PCR primers specific for bisulfite-converted DNA, and the resulting product is isolated and used as a template for methylation analysis at the CpG site(s) of interest. Small amounts of DNA can be analyzed (*e.g.*, microdissected pathology sections), and it avoids utilization of restriction enzymes for determining the methylation status at CpG sites.

Typical reagents (*e.g.*, as might be found in a typical Ms-SNuPE-based kit) for Ms-SNuPE analysis may include, but are not limited to: PCR primers for specific gene (or methylation-altered DNA sequence or CpG island); optimized PCR buffers and deoxynucleotides; gel extraction kit; positive control primers; Ms-SNuPE primers for specific gene; reaction buffer (for the Ms-SNuPE reaction); and radioactive nucleotides. Additionally, bisulfite conversion reagents may include: DNA denaturation buffer; sulfonation buffer; DNA recovery regents or kit (*e.g.*, precipitation, ultrafiltration, affinity column); desulfonation buffer; and DNA recovery components.

MSP. MSP (methylation-specific PCR) allows for assessing the methylation status of virtually any group of CpG sites within a CpG island, independent of the use of methylation-

sensitive restriction enzymes (Herman et al. *Proc. Natl. Acad. Sci. USA* 93:9821-9826, 1996; US Patent No. 5,786,146). Briefly, DNA is modified by sodium bisulfite converting all unmethylated, but not methylated cytosines to uracil, and subsequently amplified with primers specific for methylated versus unmethylated DNA. MSP requires only small quantities of DNA, is sensitive to 0.1% methylated alleles of a given CpG island locus, and can be performed on DNA extracted from paraffin-embedded samples. Typical reagents (*e.g.*, as might be found in a typical MSP-based kit) for MSP analysis may include, but are not limited to: methylated and unmethylated PCR primers for specific gene (or methylation-altered DNA sequence or CpG island), optimized PCR buffers and deoxynucleotides, and specific probes.

MCA. The MCA technique is a method that can be used to screen for altered methylation patterns in genomic DNA, and to isolate specific sequences associated with these changes (Toyota et al., *Cancer Res.* 59:2307-12, 1999). Briefly, restriction enzymes with different sensitivities to cytosine methylation in their recognition sites are used to digest genomic DNAs from primary tumors, cell lines, and normal tissues prior to arbitrarily primed PCR amplification. Fragments that show differential methylation are cloned and sequenced after resolving the PCR products on high-resolution polyacrylamide gels. The cloned fragments are then used as probes for Southern analysis to confirm differential methylation of these regions. Typical reagents (*e.g.*, as might be found in a typical MCA-based kit) for MCA analysis may include, but are not limited to: PCR primers for arbitrary priming Genomic DNA; PCR buffers and nucleotides, restriction enzymes and appropriate buffers; gene-hybridization oligos or probes; control hybridization oligos or probes.

GENOMIC SEQUENCES ACCORDING TO SEQ ID NO:1 to SEQ ID NO:58, AND TREATED VARIANTS THEREOF ACCORDING TO SEQ ID NO:304 to SEQ ID NO:535, WERE DETERMINED TO HAVE UTILITY FOR THE DETECTION, CLASSIFICATION AND/OR TREATMENT OF COLON CELL PROLIFERATIVE DISORDERS

The present invention is based upon the analysis of methylation levels within one or more genomic sequences taken from the group consisting SEQ ID NO:1 to SEQ ID NO:58.

Particular embodiments of the present invention provide a novel application of the analysis of methylation levels and/or patterns within said sequences that enables a precise

detection, characterisation and/or treatment of colon cell proliferative disorders. Early detection of colon cell proliferative disorders is directly linked with disease prognosis, and the disclosed method thereby enables the physician and patient to make better and more informed treatment decisions.

FURTHER IMPROVEMENTS

The present invention provides novel uses for genomic sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:58. Additional embodiments provide modified variants of SEQ ID NO:1 to SEQ ID NO:58, as well as oligonucleotides and/or PNA-oligomers for analysis of cytosine methylation patterns within SEQ ID NO:1 to SEQ ID NO:58.

An objective of the invention comprises analysis of the methylation state of one or more CpG dinucleotides within at least one of the genomic sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO: 58 and sequences complementary thereto.

The disclosed invention provides treated nucleic acids, derived from genomic SEQ ID NO:1 to SEQ ID NO:58, wherein the treatment is suitable to convert at least one unmethylated cytosine base of the genomic DNA sequence to uracil or another base that is detectably dissimilar to cytosine in terms of hybridization. The genomic sequences in question may comprise one, or more, consecutive or random methylated CpG positions. Said treatment preferrably comprises use of a reagent selected from the group consisting of bisulfite, hydrogen sulfite, disulfite, and combinations thereof. In a preferred embodiment of the invention, the objective comprises analysis of a modified nucleic acid comprising a sequence of at least 16 contiguous nucleotide bases in length of a sequence selected from the group consisting of SEQ ID NO:304 to SEQ ID NO:535, wherein said sequence comprises at least one CpG, TpA or CpA dinucleotide and sequences complementary thereto. The sequences of SEQ ID NO:304 to SEQ ID NO:535 provide modified versions of the nucleic acid according to SEQ ID NO:1 to SEQ ID NO:58, wherein the modification of each genomic sequence results in the synthesis of a nucleic acid having a sequence that is unique and distinct from said genomic sequence as follows. For each sense strand genomic DNA, *e.g.*,

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SEQ ID NO:1, four converted versions are disclosed. A first version wherein "C" →"T," but "CpG" remains "CpG" (*i.e.*, corresponds to case where, for the genomic sequence, all "C" residues of CpG dinucleotide sequences are methylated and are thus not converted); a second version discloses the complement of the disclosed genomic DNA sequence (i.e. *anti*sense strand), wherein "C" →"T," but "CpG" remains "CpG" (*i.e.*, corresponds to case where, for all "C" residues of CpG dinucleotide sequences are methylated and are thus not converted). The 'upmethylated' converted sequences of SEQ ID NO:1 to SEQ ID NO:58 correspond to SEQ ID NO:304 to SEQ ID NO:419. A third chemically converted version of each genomic sequences is provided, wherein "C" →"T" for all "C" residues, including those of "CpG" dinucleotide sequences (*i.e.*, corresponds to case where, for the genomic sequences, all "C" residues of CpG dinucleotide sequences are *un*methylated); a final chemically converted version of each sequence, discloses the complement of the disclosed genomic DNA sequence (i.e. *anti*sense strand), wherein "C" →"T" for all "C" residues, including those of "CpG" dinucleotide sequences (*i.e.*, corresponds to case where, for the complement (*anti*sense strand) of each genomic sequence, all "C" residues of CpG dinucleotide sequences are *un*methylated). The 'downmethylated' converted sequences of SEQ ID NO:1 to SEQ ID NO:58 correspond to SEQ ID NO:420 to SEQ ID NO:535.

Significantly, heretofore, the nucleic acid sequences and molecules according to SEQ ID NOS:1 to SEQ ID NO:535 were not implicated in or connected with the detection, classification or treatment of colon cell proliferative disorders.

In an alternative preferred embodiment, such analysis comprises the use of an oligonucleotide or oligomer for detecting the cytosine methylation state within genomic or pretreated (chemically modified) DNA, according to SEQ ID NOS:1 to SEQ ID NO:535. Said oligonucleotide or oligomer comprising a nucleic acid sequence having a length of at least nine (9) nucleotides which hybridizes, under moderately stringent or stringent conditions (as defined herein above), to a pretreated nucleic acid sequence according to SEQ ID NOS:304 to SEQ ID NO:535 and/or sequences complementary thereto, or to a genomic sequence according to SEQ ID NOS:1 to SEQ ID NO:58 and/or sequences complementary thereto.

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Thus, the present invention includes nucleic acid molecules (*e.g.*, oligonucleotides and peptide nucleic acid (PNA) molecules (PNA-oligomers)) that hybridize under moderately stringent and/or stringent hybridization conditions to all or a portion of the sequences SEQ ID NOS:1 to SEQ ID NO:535, or to the complements thereof. The hybridizing portion of the hybridizing nucleic acids is typically at least 9, 15, 20, 25, 30 or 35 nucleotides in length. However, longer molecules have inventive utility, and are thus within the scope of the present invention.

Preferably, the hybridizing portion of the inventive hybridizing nucleic acids is at least 95%, or at least 98%, or 100% identical to the sequence, or to a portion thereof of SEQ ID NOS:1 to SEQ ID NO:535, or to the complements thereof.

Hybridizing nucleic acids of the type described herein can be used, for example, as a primer (*e.g.*, a PCR primer), or a diagnostic and/or prognostic probe or primer. Preferably, hybridization of the oligonucleotide probe to a nucleic acid sample is performed under stringent conditions and the probe is 100% identical to the target sequence. Nucleic acid duplex or hybrid stability is expressed as the melting temperature or Tm, which is the temperature at which a probe dissociates from a target DNA. This melting temperature is used to define the required stringency conditions.

For target sequences that are related and substantially identical to the corresponding sequence of SEQ ID NOS:1 to SEQ ID NO:58 (such as allelic variants and SNPs), rather than identical, it is useful to first establish the lowest temperature at which only homologous hybridization occurs with a particular concentration of salt (*e.g.*, SSC or SSPE). Then, assuming that 1% mismatching results in a 1°C decrease in the Tm, the temperature of the final wash in the hybridization reaction is reduced accordingly (for example, if sequences having > 95% identity with the probe are sought, the final wash temperature is decreased by 5°C). In practice, the change in Tm can be between 0.5°C and 1.5°C per 1% mismatch.

Examples of inventive oligonucleotides of length X (in nucleotides), as indicated by polynucleotide positions with reference to, *e.g.*, SEQ ID NO:1, include those corresponding to sets (sense and antisense sets) of consecutively overlapping oligonucleotides of length X,

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where the oligonucleotides within each consecutively overlapping set (corresponding to a given X value) are defined as the finite set of Z oligonucleotides from nucleotide positions:

n to (n + (X-1));

where n=1, 2, 3,…(Y-(X-1));

where Y equals the length (nucleotides or base pairs) of SEQ ID NO:1 (2,280);

where X equals the common length (in nucleotides) of each oligonucleotide in the set (*e.g.*, X=20 for a set of consecutively overlapping 20-mers); and

where the number (Z) of consecutively overlapping oligomers of length X for a given SEQ ID NO of length Y is equal to Y-(X-1). For example Z= 2,280-19= 2,261 for either sense or antisense sets of SEQ ID NO:1, where X=20.

Preferably, the set is limited to those oligomers that comprise at least one CpG, TpG or CpA dinucleotide.

Examples of inventive 20-mer oligonucleotides include the following set of 2,261 oligomers (and the antisense set complementary thereto), indicated by polynucleotide positions with reference to SEQ ID NO:1:

1-20, 2-21, 3-22, 4-23, 5-24, ……2259-2278, 2260-2279 and 2261-2280.

Preferably, the set is limited to those oligomers that comprise at least one CpG, TpG or CpA dinucleotide.

Likewise, examples of inventive 25-mer oligonucleotides include the following set of 2,256 oligomers (and the antisense set complementary thereto), indicated by polynucleotide positions with reference to SEQ ID NO:1:

1-25, 2-26, 3-27, 4-28, 5-29, ……2254-2278, 2255-2279 and 2256-2280.

Preferably, the set is limited to those oligomers that comprise at least one CpG, TpG or CpA dinucleotide.

The present invention encompasses, for *each* of SEQ ID NOS:1 to SEQ ID NO:535 (sense and antisense), multiple consecutively overlapping sets of oligonucleotides or modified oligonucleotides of length X, where, *e.g.*, X= 9, 10, 17, 20, 22, 23, 25, 27, 30 or 35 nucleotides.

The oligonucleotides or oligomers according to the present invention constitute effective tools useful to ascertain genetic and epigenetic parameters of the genomic sequence corresponding to SEQ ID NOS:1 to SEQ ID NO:58. Preferred sets of such oligonucleotides or modified oligonucleotides of length X are those consecutively overlapping sets of oligomers corresponding to SEQ ID NOS:1 to SEQ ID NO:535 (and to the complements thereof). Preferably, said oligomers comprise at least one CpG, TpG or CpA dinucleotide.

Particularly preferred oligonucleotides or oligomers according to the present invention are those in which the cytosine of the CpG dinucleotide (or of the corresponding converted TpG or CpA dinculeotide) sequences is within the middle third of the oligonucleotide; that is, where the oligonucleotide is, for example, 13 bases in length, the CpG, TpG or CpA dinucleotide is positioned within the fifth to ninth nucleotide from the 5'-end.

The oligonucleotides of the invention can also be modified by chemically linking the oligonucleotide to one or more moieties or conjugates to enhance the activity, stability or detection of the oligonucleotide. Such moieties or conjugates include chromophores, fluorophors, lipids such as cholesterol, cholic acid, thioether, aliphatic chains, phospholipids, polyamines, polyethylene glycol (PEG), palmityl moieties, and others as disclosed in, for example, United States Patent Numbers 5,514,758, 5,565,552, 5,567,810, 5,574,142, 5,585,481, 5,587,371, 5,597,696 and 5,958,773. The probes may also exist in the form of a PNA (peptide nucleic acid) which has particularly preferred pairing properties. Thus, the oligonucleotide may include other appended groups such as peptides, and may include hybridization-triggered cleavage agents (Krol et al., *BioTechniques* 6:958-976, 1988) or intercalating agents (Zon, *Pharm. Res.* 5:539-549, 1988). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a chromophore, fluorophor, peptide, hybridization-triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The oligonucleotide may also comprise at least one art-recognized modified sugar and/or base moiety, or may comprise a modified backbone or non-natural internucleoside linkage.

The oligonucleotides or oligomers according to particular embodiments of the present invention are typically used in 'sets,' which contain at least one oligomer for analysis of each of the CpG dinucleotides of genomic sequences SEQ ID NOS:1 to SEQ ID NO:58 and sequences complementary thereto, or to the corresponding CpG, TpG or CpA dinucleotide within a sequence of the pretreated nucleic acids according to SEQ ID NOS:304 to SEQ ID NO:535 and sequences complementary thereto. However, it is anticipated that for economic or other factors it may be preferable to analyze a limited selection of the CpG dinucleotides within said sequences, and the content of the set of oligonucleotides is altered accordingly.

Therefore, in particular embodiments, the present invention provides a set of at least two (2) (oligonucleotides and/or PNA-oligomers) useful for detecting the cytosine methylation state in pretreated genomic DNA (SEQ ID NOS:304 to SEQ ID NO:535), or in genomic DNA (SEQ ID NOS:1 to SEQ ID NO:58 and sequences complementary thereto). These probes enable diagnosis, classification and/or therapy of genetic and epigenetic parameters of colon cell proliferative disorders. The set of oligomers may also be used for detecting single nucleotide polymorphisms (SNPs) in pretreated genomic DNA (SEQ ID NOS:304 to SEQ ID NO:535), or in genomic DNA (SEQ ID NOS:1 to SEQ ID NO:58 and sequences complementary thereto).

In preferred embodiments, at least one, and more preferably all members of a set of oligonucleotides is bound to a solid phase.

In further embodiments, the present invention provides a set of at least two (2) oligonucleotides that are used as 'primer' oligonucleotides for amplifying DNA sequences of one of SEQ ID NOS:1 to SEQ ID NO:535 and sequences complementary thereto, or segments thereof.

It is anticipated that the oligonucleotides may constitute all or part of an "array" or "DNA chip" (*i.e.*, an arrangement of different oligonucleotides and/or PNA-oligomers bound to a solid phase). Such an array of different oligonucleotide- and/or PNA-oligomer sequences can be characterized, for example, in that it is arranged on the solid phase in the form of a rectangular or hexagonal lattice. The solid-phase surface may be composed of silicon, glass, polystyrene, aluminum, steel, iron, copper, nickel, silver, or gold. Nitrocellulose as well as

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plastics such as nylon, which can exist in the form of pellets or also as resin matrices, may also be used. An overview of the Prior Art in oligomer array manufacturing can be gathered from a special edition of Nature Genetics (*Nature Genetics Supplement*, Volume 21, January 1999, and from the literature cited therein). Fluorescently labeled probes are often used for the scanning of immobilized DNA arrays. The simple attachment of Cy3 and Cy5 dyes to the 5'-OH of the specific probe are particularly suitable for fluorescence labels. The detection of the fluorescence of the hybridized probes may be carried out, for example, via a confocal microscope. Cy3 and Cy5 dyes, besides many others, are commercially available.

It is also anticipated that the oligonucleotides, or particular sequences thereof, may constitute all or part of an "virtual array" wherein the oligonucleotides, or particular sequences thereof, are used, for example, as 'specifiers' as part of, or in combination with a diverse population of unique labeled probes to analyze a complex mixture of analytes. Such a method, for example is described in US 2003/0013091 (United States serial number 09/898,743, published 16 January 2003). In such methods, enough labels are generated so that each nucleic acid in the complex mixture (*i.e.*, each analyte) can be uniquely bound by a unique label and thus detected (each label is directly counted, resulting in a digital read-out of each molecular species in the mixture).

It is particularly preferred that the oligomers according to the invention are utilised for at least one of: detection of; detection and differentiation between or among subclasses of; diagnosis of; prognosis of; treatment of; monitoring of; and treatment and monitoring of colon cell proliferative disorders. This is enabled by use of said sets for the detection or detection and differentiation of one or more of the following classes of tissues: colorectal carcinoma, colon adenoma, inflammatory colon tissue, grade 2 dysplasia colon adenomas less than 1 cm, grade 3 dysplasia colon adenomas larger than 1 cm, normal colon tissue, non-colon healthy tissue and non-colon cancer tissue.

Particularly preferrred are those sets of oligomer that comprise at least two oligonucleotides selected from one of the following sets of oligonucleotides:

SEQ ID NOS:59 - 285;

SEQ ID NOS:59 - 109, 113 - 223, 227 - 293;

SEQ ID NOS:59-109, 113-161, 164-223, 227-285, 287-293;

SEQ ID NOS:89, 90, 126-135, 147-151, 224-226, 253-256, 261-267, 283-285;

SEQ ID NOS:59-161, 164-293;

SEQ ID NOS:59-109, 113-299;

SEQ ID NOS:59-109, 113-293, 296–299;

SEQ ID NOS:1-12, 15-20, 22, 25-36, 38-49, 51-58;

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003, 1010 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142;

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 885, 890 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 -

915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 -
944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972,
973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 -
994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003,
1010 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034,
1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061,
1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074,
1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 -
1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114,
1115, 1115 - 1119, 1119, 1120, 1120 - 1124, 1129 - 1141, 1141, 1142, 1142;

SEQ ID NOS:738 - 740, 810 - 814, 814, 815, 815 - 829, 854 - 865, 1004 - 1006, 1006,
1007, 1007 - 1009, 1062, 1062, 1063, 1063 - 1069, 1078, 1078, 1079, 1079 - 1084, 1084,
1085, 1085, 1086, 1086, 1087, 1087 - 1091, 1121 - 1124;

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691,
691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726,
726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 784, 784, 785, 785, 786,
786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837,
837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892,
892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916,
916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957,
957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974,
974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995,
995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006,
1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 -
1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060,
1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074,
1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087
- 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114,
1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142 - 1145, 1145, 1146, 1146,

1147, 1147, 1148, 1148 - 1151, 1151, 1152, 1152 - 1154, 1154, 1155, 1155, 1156, 1156, 1157, 1157, 1158, 1158, 1159, 1159;

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 885, 890 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142;

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 -

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1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142 - 1145, 1145, 1146, 1146, 1147, 1147, 1148, 1148 - 1151, 1151, 1152, 1152;

and SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142, 1145, 1145, 1146, 1146, 1147, 1147, 1148, 1148 - 1151, 1151, 1152, 1152.

In one embodiment of the method, at least one of colorectal carcinoma tissue or colon adenomas is distinguished from at least one tissue selected from the group consisting of inflammatory colon tissue and normal colon tissue, by use of a set comprising of at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 285; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1124.

In one embodiment of the method, colorectal carcinoma is distinguished from at least one tissue selected from the group consisting of non-colon healthy tissue, peripheral blood lymphocytes and non-colon cancer of by use of a set comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 285; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974,

974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 – 1124.

In one embodiment of the method, the differentiation of is enabled by use of a set comprising of at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 109, 113 - 223, 227 - 293; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003, 1010 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142.

In one embodiment of the method, colorectal carcinoma is distinguished from at least one tissue selected from the group consisting of inflammatory colon tissue, normal colon

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tissue, non-colon healthy tissue, peripheral blood lymphocytes, colon adenomas and non-colon cancer tissue. by use of a set of oligomers comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 109, 113 - 161, 164 - 223, 227 - 285, 287 - 293; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 885, 890 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003, 1010 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1124, 1129 - 1141, 1141, 1142, 1142.

In one embodiment of the method, the colorectal carcinoma is distinguished from colon adenomas by use of a set of oligomers comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:89, 90, 126 - 135, 147 - 151, 224 - 226, 253 - 256, 261 - 267, 283 - 285; and

SEQ ID NOS:738 - 740, 810 - 814, 814, 815, 815 - 829, 854 - 865, 1004 - 1006, 1006, 1007, 1007 - 1009, 1062, 1062, 1063, 1063 - 1069, 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1091, 1121 - 1124.

In one embodiment of the method, at least one of colorectal carcinoma tissue, or colon adenomas is distinguished from at least one tissue selected from the group consisting of inflammatory colon tissue and normal colon tissue is enabled by use of a set of oligomers comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 303; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142 - 1145, 1145, 1146, 1146, 1147, 1147, 1148, 1148 - 1151, 1151, 1152, 1152 - 1154, 1154, 1155, 1155, 1156, 1156, 1157, 1157, 1158, 1158, 1159, 1159.

In one embodiment of the method, colorectal carcinoma tissue is distinguished from at least one of inflammatory colon tissue and normal colon tissue by use of a set of oligomers comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 161, 164 - 293; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726,

726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 885, 890 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142.

In one embodiment of the method, at least one of colorectal carcinoma tissue, or colon adenomas is distinguished from at least one tissue selected from the group consisting of inflammatory colon tissue, normal colon tissue, non-colon healthy tissue, peripheral blood lymphocytes, and non-colon cancer tissue by use of a set of oligomers comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ IDNOS:59 - 109, 113 - 299; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994,

995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142 - 1145, 1145, 1146, 1146, 1147, 1147, 1148, 1148 - 1151, 1151, 1152, 1152.

In one embodiment of the method, tissues originating from the colon are distinguished from tissues of non-colon origin by use of a set of oligomers comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 303; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142 - 1145, 1145, 1146, 1146,

1147, 1147, 1148, 1148 - 1151, 1151, 1152, 1152 - 1154, 1154, 1155, 1155, 1156, 1156, 1157, 1157, 1158, 1158, 1159, 1159.

In one embodiment of the method, cell proliferative disorders are distinguished from healthy tissues by use of a set of oligomers comprising at least two oligonucleotides selected from one of the groups consisting of:

SEQ ID NOS:59 - 109, 113 - 293, 296 - 299; and

SEQ ID NOS:681 - 683, 683, 684, 684, 685, 685, 686, 686, 687, 687, 688, 688 - 691, 691, 692, 692 - 695, 695, 696, 696 - 699, 699, 700, 700 - 709, 709, 710, 710 - 725, 725, 726, 726, 727, 727, 728, 728 - 760, 760, 761, 761, 762, 762, 763, 763 - 777, 784, 784, 785, 785, 786, 786, 787, 787 - 802, 802, 803, 803 - 814, 814, 815, 815 - 832, 832, 833, 833 - 836, 836, 837, 837 - 872, 872, 873, 873 - 876, 876, 877, 877, 878, 878, 879, 879 - 882, 882, 883, 883 - 892, 892, 893, 893 - 896, 896, 897, 897 - 909, 909, 910, 910, 911, 911, 912, 912 - 915, 915, 916, 916 - 921, 921 - 925, 925, 926, 926 - 939, 939, 940, 940, 941, 941, 942, 942 - 944, 944 - 957, 957, 958, 958, 959, 959, 960, 960, 961, 961, 962, 962 - 971, 971, 972, 972, 973, 973, 974, 974, 975, 975, 976, 976 - 979, 979, 980, 980, 981, 981 - 988, 988, 989, 989 - 994, 994, 995, 995 - 998, 998, 999, 999, 1000, 1000, 1001, 1001, 1002, 1002, 1003, 1003 - 1006, 1006, 1007, 1007 - 1016, 1016, 1017, 1017, 1018, 1018, 1019, 1019 - 1028, 1028, 1029, 1029 - 1034, 1034, 1035, 1035 - 1046, 1046, 1047, 1047 - 1058, 1058, 1059, 1059, 1060, 1060, 1061, 1061, 1062, 1062, 1063, 1063 - 1070, 1070, 1071, 1071, 1072, 1072, 1073, 1073, 1074, 1074, 1075, 1075 - 1078, 1078, 1079, 1079 - 1084, 1084, 1085, 1085, 1086, 1086, 1087, 1087 - 1092, 1092, 1093, 1093, 1094, 1094, 1095, 1095 - 1102, 1102, 1103, 1103 - 1114, 1114, 1115, 1115 - 1119, 1119, 1120, 1120 - 1141, 1141, 1142, 1142, 1145, 1145, 1146, 1146, 1147, 1147, 1148, 1148 - 1151, 1151, 1152, 1152.

The present invention further provides a method for ascertaining genetic and/or epigenetic parameters of the genomic sequences according to SEQ ID NOS:1 to SEQ ID NO:58 within a subject by analyzing cytosine methylation and single nucleotide polymorphisms. Said method comprising contacting a nucleic acid comprising one or more of SEQ ID NOS:1 to SEQ ID NO:58 in a biological sample obtained from said subject with at least one reagent or a series of reagents, wherein said reagent or series of reagents,

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distinguishes between methylated and non-methylated CpG dinucleotides within the target nucleic acid.

Preferably, said method comprises the following steps: In the *first step*, a sample of the tissue to be analysed is obtained. The source may be any suitable source, such as cell lines, histological slides, biopsies, tissue embedded in paraffin, bodily fluids, ejaculate, urine, blood and all possible combinations thereof. The DNA is then isolated from the sample. Extraction may be by means that are standard to one skilled in the art, including the use of commercially available kits, detergent lysates, sonification and vortexing with glass beads. Once the nucleic acids have been extracted, the genomic double stranded DNA is used in the analysis.

In the *second step* of the method, the genomic DNA sample is treated in such a manner that cytosine bases which are unmethylated at the 5'-position are converted to uracil, thymine, or another base which is dissimilar to cytosine in terms of hybridization behavior. This will be understood as 'pretreatment' or 'treatment' herein.

The above-described treatment of genomic DNA is preferably carried out with bisulfite (hydrogen sulfite, disulfite) and subsequent alkaline hydrolysis which results in a conversion of non-methylated cytosine nucleobases to uracil or to another base which is dissimilar to cytosine in terms of base pairing behavior.

In the *third step* of the method, fragments of the pretreated DNA are amplified, using sets of primer oligonucleotides according to the present invention, and an amplification enzyme. The amplification of several DNA segments can be carried out simultaneously in one and the same reaction vessel. Typically, the amplification is carried out using a polymerase chain reaction (PCR). The set of primer oligonucleotides includes at least two oligonucleotides whose sequences are each reverse complementary, identical, or hybridize under stringent or highly stringent conditions to an at least 16-base-pair long segment of the base sequences of one or more of SEQ ID NOS:304 to SEQ ID NO:535 and sequences complementary thereto.

In an alternate embodiment of the method, the methylation status of preselected CpG positions within the nucleic acid sequences comprising one or more of SEQ ID NOS:1 to SEQ ID NO:58 may be detected by use of methylation-specific primer oligonucleotides. This

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technique (MSP) has been described in United States Patent No. 6,265,171 to Herman. The use of methylation status specific primers for the amplification of bisulfite treated DNA allows the differentiation between methylated and unmethylated nucleic acids. MSP primers pairs contain at least one primer which hybridizes to a bisulfite treated CpG dinucleotide. Therefore, the sequence of said primers comprises at least one CpG dinucleotide. MSP primers specific for non-methylated DNA contain a "T' at the 3' position of the C position in the CpG. Preferably, therefore, the base sequence of said primers is required to comprise a sequence having a length of at least 9 nucleotides which hybridizes to a pretreated nucleic acid sequence according to one of SEQ ID NOS:304 to SEQ ID NO:535 and sequences complementary thereto, wherein the base sequence of said oligomers comprises at least one CpG dinucleotide.

In a further preferred embodiment of the method, the MSP primers are selected from the group consisting SEQ ID NOS:2754, 2757, 2759, 2767, 2768, 2772, 2776, 2779, 2784, 2785, 2786, 2789, 2791, 2792, 2794, 2797, 2798, 2801, 2805, 2806, 2809, 2811, 2815, 2817, 2824, 2830, 2832, 2833, 2834, 2838, 2840, 2843, 2846, 2850, 2851, 2853, 2855, 2859, 2866, 2867, 2870, 2871, 2876, 2878, 2881, 2885, 2886, 2892, 2893, 2895, 2896, 2898, 2904, 2912, 2915, 2917, 2919, 2922, 2923, 2929, 2931, 2939, 2941, 2944, 2946, 2948, 2950, 2955, 2956, 2957, 2960, 2962, 2965, 2966, 2967, 2968, 2969, 2971, 2975, 2976, 2977, 2978, 2979, 2980, 2981, 2984, 2988, 2989, 2990, 2992, 2994, 2997, 2999, 3001, 3002, 3004, 3006, 3007, 3008, 3010, 3013, 3014, 3016, 3017, 3018, 3020, 3022, 3025, 2752, 2755, 2760, 2762, 2763, 2764, 2765, 2769, 2771, 2773, 2775, 2777, 2780, 2781, 2782, 2783, 2787, 2788, 2790, 2793, 2795, 2796, 2800, 2802, 2803, 2804, 2808, 2810, 2812, 2814, 2816, 2818, 2820, 2821, 2823, 2825, 2827, 2828, 2829, 2831, 2835, 2836, 2837, 2839, 2841, 2842, 2844, 2845, 2847, 2849, 2852, 2854, 2856, 2858, 2860, 2861, 2862, 2863, 2864, 2865, 2868, 2869, 2872, 2873, 2874, 2875, 2877, 2879, 2880, 2882, 2883, 2884, 2887, 2888, 2889, 2890, 2891, 2894, 2897, 2899, 2901, 2902, 2903, 2905, 2907, 2909, 2911, 2914, 2916, 2918, 2920, 2924, 2926, 2927, 2928, 2930, 2932, 2933, 2934, 2935, 2936, 2937, 2938, 2942, 2945, 2947, 2949, 2951, 2953, 2954, 2958, 2959, 2961, 2963, 2970, 2972, 2973, 2974, 2982, 2985, 2987, 2991, 2993, 2995, 2996, 2998, 3000, 3003, 3005, 3011, 3019, 3021, 3023, 3026, 3027, 3028, 3032, 3033, 3036, 3037, 3038,

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3784, 3788, 3792, 3794, 3800, 3801, 3802, 3803, 3804, 3806, 3807, 3808, 3810, 3812, 3813,

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A further preferred embodiment of the method comprises the use of *blocker* oligonucleotides. The use of such blocker oligonucleotides has been described by Yu et al., *BioTechniques* 23:714-720, 1997. Blocking probe oligonucleotides are hybridized to the bisulfite treated nucleic acid concurrently with the PCR primers. PCR amplification of the nucleic acid is terminated at the 5' position of the blocking probe, such that amplification of a nucleic acid is suppressed where the complementary sequence to the blocking probe is present. The probes may be designed to hybridize to the bisulfite treated nucleic acid in a methylation status specific manner. For example, for detection of methylated nucleic acids within a population of unmethylated nucleic acids, suppression of the amplification of nucleic acids which are unmethylated at the position in question would be carried out by the use of

blocking probes comprising a 'CpA' or 'TpA' at the position in question, as opposed to a 'CpG' if the suppression of amplification of methylated nucleic acids is desired.

For PCR methods using blocker oligonucleotides, efficient disruption of polymerase-mediated amplification requires that blocker oligonucleotides not be elongated by the polymerase. Preferably, this is achieved through the use of blockers that are 3'-deoxyoligonucleotides, or oligonucleotides derivitized at the 3' position with other than a "free" hydroxyl group. For example, 3'-O-acetyl oligonucleotides are representative of a preferred class of blocker molecule.

Additionally, polymerase-mediated decomposition of the blocker oligonucleotides should be precluded. Preferably, such preclusion comprises either use of a polymerase lacking 5'-3' exonuclease activity, or use of modified blocker oligonucleotides having, for example, thioate bridges at the 5'-terminii thereof that render the blocker molecule nuclease-resistant. Particular applications may not require such 5' modifications of the blocker. For example, if the blocker- and primer-binding sites overlap, thereby precluding binding of the primer (*e.g.*, with excess blocker), degradation of the blocker oligonucleotide will be substantially precluded. This is because the polymerase will not extend the primer toward, and through (in the 5'-3' direction) the blocker—a process that normally results in degradation of the hybridized blocker oligonucleotide.

A particularly preferred blocker/PCR embodiment, for purposes of the present invention and as implemented herein, comprises the use of peptide nucleic acid (PNA) oligomers as blocking oligonucleotides. Such PNA blocker oligomers are ideally suited, because they are neither decomposed nor extended by the polymerase.

Preferably, therefore, the base sequence of said *blocking oligonucleotides* is required to comprise a sequence having a length of at least 9 nucleotides which hybridizes to a pretreated nucleic acid sequence according to one of SEQ ID NOS:304 to SEQ ID NO:535 and sequences complementary thereto, wherein the base sequence of said oligonucleotides comprises at least one CpG, TpG or CpA dinucleotide.

In a further preferred embodiment of the method, the *blocking oligonucleotides* are selected from the group consisting of SEQ ID NOS:2114, 2119, 2124, 2125, 2127, 2132,

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The fragments obtained by means of the amplification can carry a directly or indirectly detectable label. Preferred are labels in the form of fluorescence labels, radionuclides, or detachable molecule fragments having a typical mass which can be detected in a mass spectrometer. Where said labels are mass labels, it is preferred that the labeled amplificates have a single positive or negative net charge, allowing for better detectability in the mass spectrometer. The detection may be carried out and visualized by means of, *e.g.*, matrix assisted laser desorption/ionization mass spectrometry (MALDI) or using electron spray mass spectrometry (ESI).

Matrix Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-TOF) is a very efficient development for the analysis of biomolecules (Karas & Hillenkamp, *Anal Chem.*, 60:2299-301, 1988). An analyte is embedded in a light-absorbing matrix. The matrix is evaporated by a short laser pulse thus transporting the analyte molecule into the vapour phase in an unfragmented manner. The analyte is ionized by collisions with matrix molecules. An applied voltage accelerates the ions into a field-free flight tube. Due to their different masses, the ions are accelerated at different rates. Smaller ions reach the detector sooner than bigger ones. MALDI-TOF spectrometry is well suited to the analysis of peptides and proteins. The analysis of nucleic acids is somewhat more difficult (Gut & Beck, *Current Innovations and Future Trends*, 1:147-57, 1995). The sensitivity with respect to nucleic acid analysis is approximately 100-times less than for peptides, and decreases disproportionally with increasing fragment size. Moreover, for nucleic acids having a multiply negatively charged backbone, the ionization process via the matrix is considerably less efficient. In MALDI-TOF spectrometry, the selection of the matrix plays an eminently important role. For desorption of peptides, several very efficient matrixes have been found which produce a very fine crystallisation. There are now several responsive matrixes for DNA, however, the

difference in sensitivity between peptides and nucleic acids has not been reduced. This difference in sensitivity can be reduced, however, by chemically modifying the DNA in such a manner that it becomes more similar to a peptide. For example, phosphorothioate nucleic acids, in which the usual phosphates of the backbone are substituted with thiophosphates, can be converted into a charge-neutral DNA using simple alkylation chemistry (Gut & Beck, *Nucleic Acids Res.* 23: 1367-73, 1995). The coupling of a charge tag to this modified DNA results in an increase in MALDI-TOF sensitivity to the same level as that found for peptides. A further advantage of charge tagging is the increased stability of the analysis against impurities, which makes the detection of unmodified substrates considerably more difficult.

In the *fourth step* of the method, the amplificates obtained during the third step of the method are analysed in order to ascertain the methylation status of the CpG dinucleotides prior to the treatment.

In embodiments where the amplificates were obtained by means of MSP amplification, the presence or absence of an amplificate is in itself indicative of the methylation state of the CpG positions covered by the primer, according to the base sequences of said primer.

Amplificates obtained by means of both standard and methylation specific PCR may be further analyzed by means of hybridization-based methods such as, but not limited to, array technology and probe based technologies as well as by means of techniques such as sequencing and template directed extension.

In one embodiment of the method, the amplificates synthesised in *step three* are subsequently hybridized to an array or a set of oligonucleotides and/or PNA probes. In this context, the hybridization takes place in the following manner: the set of probes used during the hybridization is preferably composed of at least 2 oligonucleotides or PNA-oligomers; in the process, the amplificates serve as probes which hybridize to oligonucleotides previously bonded to a solid phase; the non-hybridized fragments are subsequently removed; said oligonucleotides contain at least one base sequence having a length of at least 9 nucleotides which is reverse complementary or identical to a segment of the base sequences specified in

the present Sequence Listing; and the segment comprises at least one CpG , TpG or CpA dinucleotide.

In a preferred embodiment, said dinucleotide is present in the central third of the oligomer. For example, wherein the oligomer comprises one CpG dinucleotide, said dinucleotide is preferably the fifth to ninth nucleotide from the 5'-end of a 13-mer. One oligonucleotide exists for the analysis of each CpG dinucleotide within the sequence according to SEQ ID NOS:1 to SEQ ID NO:58, and the equivalent positions within SEQ ID NOS:304 to SEQ ID NO:535. Said oligonucleotides may also be present in the form of peptide nucleic acids. The non-hybridized amplificates are then removed.The hybridized amplificates are then detected. In this context, it is preferred that labels attached to the amplificates are identifiable at each position of the solid phase at which an oligonucleotide sequence is located.

In yet a further embodiment of the method, the genomic methylation status of the CpG positions may be ascertained by means of oligonucleotide probes that are hybridised to the bisulfite treated DNA concurrently with the PCR amplification primers (wherein said primers may either be methylation specific or standard).

A particularly preferred embodiment of this method is the use of fluorescence-based Real Time Quantitative PCR (Heid et al., *Genome Res.* 6:986-994, 1996; *also see* United States Patent No. 6,331,393) employing a dual-labeled fluorescent oligonucleotide probe (TaqMan™ PCR, using an ABI Prism 7700 Sequence Detection System, Perkin Elmer Applied Biosystems, Foster City, California). The TaqMan™ PCR reaction employs the use of a nonextendible interrogating oligonucleotide, called a TaqMan™ probe, which, in preferred imbodiments, is designed to hybridize to a GpC-rich sequence located between the forward and reverse amplification primers. The TaqMan™ probe further comprises a fluorescent "reporter moiety" and a "quencher moiety" covalently bound to linker moieties (*e.g.*, phosphoramidites) attached to the nucleotides of the TaqMan™ oligonucleotide. For analysis of methylation within nucleic acids subsequent to bisulfite treatment, it is required that the probe be methylation specific, as described in United States Patent No. 6,331,393, (hereby incorporated by reference in its entirety) also known as the MethylLight™ assay.

Variations on the TaqMan™ detection methodology that are also suitable for use with the described invention include the use of dual-probe technology (Lightcycler™) or fluorescent amplification primers (Sunrise™ technology). Both these techniques may be adapted in a manner suitable for use with bisulfite treated DNA, and moreover for methylation analysis within CpG dinucleotides.

A further suitable method for the use of probe oligonucleotides for the assessment of methylation by analysis of bisulfite treated nucleic acids In a further preferred embodiment of the method, the *fifth step* of the method comprises the use of template-directed oligonucleotide extension, such as MS-SNuPE as described by Gonzalgo & Jones, *Nucleic Acids Res.* 25:2529-2531, 1997.

In yet a further embodiment of the method, the *fifth step* of the method comprises sequencing and subsequent sequence analysis of the amplificate generated in the *third step* of the method (Sanger F., et al., *Proc Natl Acad Sci USA* 74:5463-5467, 1977).

Best mode

In the most preferred embodiment of the method the nucleic acids according to SEQ ID NO: 1 to SEQ ID NO 58 are isolated and treated according to the first three steps of the method outlined above, namely:

a)   obtaining, from a subject, a biological sample having subject genomic DNA;

b)   extracting or otherwise isolating the genomic DNA;

c)   treating the genomic DNA of b), or a fragment thereof, with one or more reagents to convert cytosine bases that are unmethylated in the 5-position thereof to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridization properties; and wherein

d)   amplifying subsequent to treatment in c) is carried out in a methylation specific manner, namely by use of methylation specific primers or *blocking oligonucleotides*, and further wherein

e)   detecting of the amplificates is carried out by means of a real-time detection probe, as described above.

Preferably, where the subsequent amplification of d) is carried out by means of methylation specific primers, as described above, said methylation specific primers comprise a sequence having a length of at least 9 nucleotides which hybridizes to a pretreated nucleic acid sequence according to one of SEQ ID NOS:304 to SEQ ID NO:535 and sequences complementary thereto, wherein the base sequence of said oligomers comprises at least one CpG dinucleotide.

In a further preferred embodiment of the method, the MSP primers are selected from the group consisting of SEQ ID NOS:2754, 2757, 2759, 2767, 2768, 2772, 2776, 2779, 2784, 2785, 2786, 2789, 2791, 2792, 2794, 2797, 2798, 2801, 2805, 2806, 2809, 2811, 2815, 2817, 2824, 2830, 2832, 2833, 2834, 2838, 2840, 2843, 2846, 2850, 2851, 2853, 2855, 2859, 2866, 2867, 2870, 2871, 2876, 2878, 2881, 2885, 2886, 2892, 2893, 2895, 2896, 2898, 2904, 2912, 2915, 2917, 2919, 2922, 2923, 2929, 2931, 2939, 2941, 2944, 2946, 2948, 2950, 2955, 2956, 2957, 2960, 2962, 2965, 2966, 2967, 2968, 2969, 2971, 2975, 2976, 2977, 2978, 2979, 2980, 2981, 2984, 2988, 2989, 2990, 2992, 2994, 2997, 2999, 3001, 3002, 3004, 3006, 3007, 3008, 3010, 3013, 3014, 3016, 3017, 3018, 3020, 3022, 3025, 2752, 2755, 2760, 2762, 2763, 2764, 2765, 2769, 2771, 2773, 2775, 2777, 2780, 2781, 2782, 2783, 2787, 2788, 2790, 2793, 2795, 2796, 2800, 2802, 2803, 2804, 2808, 2810, 2812, 2814, 2816, 2818, 2820, 2821, 2823, 2825, 2827, 2828, 2829, 2831, 2835, 2836, 2837, 2839, 2841, 2842, 2844, 2845, 2847, 2849, 2852, 2854, 2856, 2858, 2860, 2861, 2862, 2863, 2864, 2865, 2868, 2869, 2872, 2873, 2874, 2875, 2877, 2879, 2880, 2882, 2883, 2884, 2887, 2888, 2889, 2890, 2891, 2894, 2897, 2899, 2901, 2902, 2903, 2905, 2907, 2909, 2911, 2914, 2916, 2918, 2920, 2924, 2926, 2927, 2928, 2930, 2932, 2933, 2934, 2935, 2936, 2937, 2938, 2942, 2945, 2947, 2949, 2951, 2953, 2954, 2958, 2959, 2961, 2963, 2970, 2972, 2973, 2974, 2982, 2985, 2987, 2991, 2993, 2995, 2996, 2998, 3000, 3003, 3005, 3011, 3019, 3021, 3023, 3026, 3027, 3028, 3032, 3033, 3036, 3037, 3038, 3039, 3041, 3042, 3043, 3044, 3047, 3048, 3049, 3052, 3055, 3059, 3061, 3064, 3065, 3068, 3069, 3071, 3072, 3075, 3076, 3080, 3083, 3084, 3085, 3086, 3091, 3093, 3096, 3097, 3100, 3104, 3109, 3110, 3113, 3115, 3117, 3118, 3123, 3126, 3127, 3130, 3134, 3135, 3136, 3138, 3139, 3144, 3146, 3147, 3149, 3150, 3155, 3029, 3031, 3034, 3040, 3045, 3050, 3051, 3053, 3054, 3056, 3057, 3060, 3063, 3066, 3073, 3078, 3081, 3088, 3089, 3090, 3092, 3094, 3098,

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14374, 14376, 14379, 14380, 14381, 14382, 14383, 14384, and SEQ ID NO:14385.

Step e) of the method, namely the detection of the specific amplificates indicative of
the methylation status of one or more CpG positions according to SEQ ID NOS:1 to SEQ ID
NO:58 is carried out by means of *real-time* detection methods as described above, and
wherein the sequence of said hybridization probes is selected from the group consisting of
SEQ ID NOS:2753, 2756, 2758, 2761, 2766, 2770, 2774, 2778, 2799, 2807, 2813, 2819,

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ID NO:14378.

For each genomic sequence listed, the following oligonucleotides as detailed in the sequence listing may be used for a combined MSP-RealTime analysis:

Taqman Real-time single probe detection assays:

SEQ ID NO: 41

left Primer: 2751, 2754, 2757, 2759, 2767, 2768, 2772, 2776, 2779, 2784, 2785, 2786, 2789, 2791, 2792, 2794, 2797, 2798, 2801, 2805, 2806, 2809, 2811, 2815, 2817, 2824, 2830, 2832, 2833, 2834, 2838, 2840, 2843, 2846, 2850, 2851, 2853, 2855, 2859, 2866, 2867, 2870, 2871, 2876, 2878, 2881, 2885, 2886, 2892, 2893, 2895, 2896, 2898, 2904, 2912, 2915, 2917, 2919, 2922, 2923, 2929, 2931, 2939, 2941, 2944, 2946, 2948, 2950, 2955, 2956, 2957, 2960, 2962, 2965, 2966, 2967, 2968, 2969, 2971, 2975, 2976, 2977, 2978, 2979, 2980, 2981, 2984, 2988, 2989, 2990, 2992, 2994, 2997, 2999, 3001, 3002, 3004, 3006, 3007, 3008, 3010, 3013, 3014, 3016, 3017, 3018, 3020, 3022, 3025,

right Primer: 2752, 2755, 2760, 2762, 2763, 2764, 2765, 2769, 2771, 2773, 2775, 2777, 2780, 2781, 2782, 2783, 2787, 2788, 2790, 2793, 2795, 2796, 2800, 2802, 2803, 2804, 2808, 2810, 2812, 2814, 2816, 2818, 2820, 2821, 2823, 2825, 2827, 2828, 2829, 2831, 2835, 2836, 2837, 2839, 2841, 2842, 2844, 2845, 2847, 2849, 2852, 2854, 2856, 2858, 2860, 2861, 2862, 2863, 2864, 2865, 2868, 2869, 2872, 2873, 2874, 2875, 2877, 2879, 2880, 2882, 2883, 2884, 2887, 2888, 2889, 2890, 2891, 2894, 2897, 2899, 2901, 2902, 2903, 2905, 2907, 2909, 2911, 2914, 2916, 2918, 2920, 2924, 2926, 2927, 2928, 2930, 2932, 2933, 2934, 2935, 2936, 2937, 2938, 2942, 2945, 2947, 2949, 2951, 2953, 2954, 2958, 2959, 2961, 2963, 2970, 2972, 2973, 2974, 2982, 2985, 2987, 2991, 2993, 2995, 2996, 2998, 3000, 3003, 3005, 3011, 3019, 3021, 3023, 3026, 3027,

Detection: 2753, 2756, 2758, 2761, 2766, 2770, 2774, 2778, 2799, 2807, 2813, 2819, 2822, 2826, 2848, 2857, 2900, 2906, 2908, 2910, 2913, 2921, 2925, 2940, 2943, 2952, 2964, 2983, 2986, 3009, 3012, 3015, 3024,

SEQ ID NO: 5

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right Primer: 3029, 3031, 3034, 3040, 3045, 3050, 3051, 3053, 3054, 3056, 3057, 3060, 3063, 3066, 3073, 3078, 3081, 3088, 3089, 3090, 3092, 3094, 3098, 3101, 3103, 3105, 3107, 3108, 3111, 3114, 3116, 3119, 3121, 3122, 3124, 3128, 3131, 3133, 3137, 3140, 3142, 3145, 3148, 3151, 3152, 3153, 3157,

Detection: 3030, 3035, 3046, 3058, 3062, 3067, 3070, 3074, 3077, 3079, 3082, 3087, 3095, 3099, 3102, 3106, 3112, 3120, 3125, 3129, 3132, 3141, 3143, 3154, 3156, 3158,

SEQ ID NO: 6

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right Primer: 3160, 3162, 3163, 3164, 3168, 3169, 3173, 3178, 3182, 3183, 3187, 3189, 3190, 3195, 3196, 3197, 3199, 3205, 3207, 3210, 3214, 3219, 3222, 3223, 3225, 3227, 3236, 3240, 3242, 3243, 3244, 3246, 3250,

Detection: 3161, 3177, 3188, 3191, 3193, 3200, 3208, 3211, 3220, 3231, 3241, 3251,

SEQ ID NO: 8

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right Primer: 3253, 3255, 3257, 3259, 3260, 3262, 3265, 3266, 3267, 3271, 3274, 3276, 3278, 3287, 3289, 3291, 3293, 3298, 3300, 3302, 3309, 3313, 3314, 3316, 3318, 3321, 3322, 3325, 3328, 3330, 3332, 3335, 3337, 3338, 3340, 3342, 3343, 3344, 3345, 3346, 3347, 3349, 3350, 3352, 3353, 3355, 3356, 3357, 3359, 3361, 3363, 3365, 3369, 3371, 3372, 3373,

Detection: 3254, 3258, 3263, 3272, 3277, 3288, 3294, 3296, 3299, 3303, 3317, 3323, 3326, 3329, 3336, 3341, 3362, 3366, 3370, 3375,

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right Primer: 3378, 3381, 3384, 3385, 3393, 3397, 3399, 3402,

Detection: 3379, 3382, 3387, 3394, 3398,

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right Primer: 3404, 3409, 3411, 3415, 3418, 3419, 3421, 3424, 3426, 3427, 3429, 3431, 3435, 3437, 3438, 3441, 3444, 3446, 3448, 3450, 3452, 3454, 3456, 3459, 3461, 3462, 3464, 3467, 3476, 3479, 3482, 3485, 3488, 3489, 3491, 3492, 3493, 3501, 3503, 3506, 3509, 3510, 3511, 3513, 3517, 3518, 3519, 3520, 3522, 3524, 3526, 3527, 3528, 3530, 3531, 3535, 3536, 3545, 3549, 3551, 3552, 3554, 3555, 3556, 3561, 3564, 3565, 3568, 3569, 3570, 3573, 3576, 3578, 3581, 3587, 3588, 3589, 3590, 3591, 3593, 3596, 3600, 3602, 3605, 3606, 3609, 3611, 3613, 3614, 3615, 3616, 3617, 3618, 3623, 3624, 3625, 3627, 3629, 3630, 3633, 3634, 3635, 3636, 3641, 3645, 3651, 3653, 3654, 3655, 3658, 3661, 3662, 3665, 3666, 3667, 3669, 3671, 3674, 3679, 3681, 3684, 3686, 3689, 3691, 3692, 3693, 3695, 3696, 3698, 3701, 3705, 3706, 3707, 3709,

Detection: 3405, 3407, 3410, 3412, 3416, 3422, 3425, 3430, 3449, 3451, 3457, 3460, 3465, 3468, 3486, 3498, 3502, 3507, 3523, 3532, 3539, 3546, 3550, 3567, 3572, 3579, 3582, 3610, 3620, 3628, 3648, 3659, 3664, 3672, 3675, 3680, 3682, 3685, 3687, 3699,

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right Primer: 3711, 3713, 3719, 3721, 3724, 3725, 3726, 3727, 3728, 3731, 3733, 3736, 3737, 3738, 3742, 3747, 3748, 3750, 3752, 3757, 3758, 3759, 3760,

Detection: 3712, 3716, 3720, 3734, 3751,

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right Primer: 3762, 3765, 3768, 3770, 3772, 3774, 3778, 3779, 3780, 3782, 3784, 3788, 3792, 3794, 3800, 3801, 3802, 3803, 3804, 3806, 3807, 3808, 3810, 3812, 3813, 3815, 3819, 3820, 3821, 3824, 3826, 3827, 3829, 3831, 3832, 3834, 3837, 3838, 3839, 3840, 3842, 3845, 3848, 3852, 3854, 3858, 3860, 3861, 3863, 3864, 3865, 3866, 3867, 3868, 3869, 3870, 3871, 3872, 3874, 3875, 3878, 3881, 3883, 3884, 3886, 3888, 3891, 3894, 3897, 3898, 3900, 3901, 3904, 3909, 3911, 3914, 3915, 3917, 3919, 3920, 3921, 3922, 3924, 3928, 3931, 3933, 3934, 3937, 3941, 3942, 3943, 3947, 3948, 3952, 3958, 3959, 3961, 3962, 3963, 3964, 3966, 3968, 3972, 3974, 3975, 3976, 3980, 3981, 3986,

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4637,

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right Primer: 4650, 4653, 4656, 4658, 4660, 4662, 4666, 4667, 4669, 4670, 4672,

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Detection: 4651, 4654, 4657, 4673, 4679, 4682, 4692, 4698, 4710, 4712, 4713, 4723,

4725, 4729, 4734, 4740, 4743, 4747, 4752, 4757, 4762, 4765, 4775, 4781, 4796, 4799, 4803,

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SEQ ID NO: 44

left Primer: 4953,

right Primer: 4954, 4956,

Detection: 4955,

SEQ ID NO:1

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4989, 4991, 4992, 4997, 4999, 5002, 5003, 5004, 5006, 5010, 5013, 5014, 5015, 5017, 5018,

5019, 5020, 5021, 5024, 5025, 5027, 5031, 5032, 5033, 5037, 5040, 5041, 5042, 5043,

right Primer: 4958, 4961, 4964, 4966, 4968, 4969, 4973, 4974, 4975, 4978, 4979, 4980, 4981, 4982, 4983, 4984, 4988, 4990, 4993, 4995, 4996, 4998, 5000, 5005, 5007, 5009, 5011, 5016, 5022, 5026, 5028, 5030, 5034, 5036, 5038, 5039,

Detection: 4959, 4962, 4965, 4994, 5001, 5008, 5012, 5023, 5029, 5035,

SEQ ID NO:2

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right Primer: 5045, 5048, 5051, 5053, 5056, 5058, 5062, 5065,

Detection: 5046, 5059, 5063,

SEQ ID NO: 45

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right Primer: 7556, 7561, 7563, 7568, 7583, 7584, 7591, 7593, 7594, 7595, 7599, 7601, 7604, 7607, 7608, 7611, 7613, 7614, 7616, 7617, 7619, 7622, 7624, 7626, 7628, 7631, 7637, 7638, 7639, 7640, 7641, 7642, 7644, 7645, 7646, 7649, 7650, 7652, 7654, 7656, 7657, 7658, 7659, 7660, 7662, 7663, 7665, 7667, 7669, 7670, 7671, 7673, 7675, 7678, 7679, 7680, 7681, 7684, 7686, 7688, 7692, 7695, 7697, 7698, 7700, 7703, 7705, 7708, 7710, 7715, 7717, 7719, 7721, 7722, 7723, 7724, 7725, 7727, 7730, 7731, 7732, 7737, 7739, 7742, 7743, 7745, 7757, 7759, 7760,

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Detection: 7557, 7562, 7564, 7569, 7572, 7592, 7602, 7605, 7612, 7625, 7630, 7632, 7634, 7648, 7653, 7664, 7668, 7693, 7696, 7706,

SEQ ID NO: 20

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right Primer: 7762, 7765, 7767, 7768, 7771, 7773, 7776, 7779, 7781, 7783, 7784, 7785, 7789, 7791, 7792, 7793, 7794, 7796, 7798, 7800, 7802, 7803, 7808, 7812, 7813, 7814, 7816, 7818, 7821, 7825, 7827, 7829, 7830, 7833, 7835, 7836, 7838, 7840, 7841, 7842, 7844, 7846, 7847, 7848, 7849, 7853, 7854, 7855, 7856, 7858, 7861, 7864, 7866, 7869, 7870, 7871, 7872, 7873, 7875, 7877, 7878, 7879, 7880, 7882, 7884, 7886, 7888, 7890, 7897, 7899, 7903, 7905, 7912, 7914, 7916, 7918, 7919, 7920, 7921, 7923, 7929, 7931, 7932, 7936, 7938, 7939, 7940, 7942, 7943, 7944, 7947, 7949, 7958, 7960, 7962, 7965, 7967, 7969, 7970, 7974, 7977, 7989, 7990,

Detection: 7763, 7766, 7769, 7772, 7777, 7780, 7787, 7790, 7801, 7804, 7806, 7826, 7831, 7850, 7859, 7862, 7867, 7876, 7883, 7887, 7891, 7893, 7895, 7898, 7901, 7906, 7908, 7911, 7915, 7924, 7927, 7930, 7935, 7959, 7961, 7966, 7975, 7978, 7984,

SEQ ID NO: 48

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right Primer: 7993, 7997, 7999, 8001, 8004, 8005, 8008, 8009, 8011, 8013, 8017, 8020, 8021, 8022, 8023, 8024, 8025, 8028, 8031, 8033, 8036, 8040, 8042, 8047, 8051, 8054, 8057, 8059, 8060, 8061, 8065, 8072, 8073, 8075, 8082, 8083, 8084, 8085, 8087, 8088, 8089

Detection: 7994, 7998, 8002, 8014, 8029, 8032, 8037, 8041, 8045, 8048, 8058, 8063, 8086,

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right Primer: 8091, 8094, 8096, 8099, 8103, 8105, 8108, 8112, 8113, 8117, 8119, 8121, 8123, 8125, 8128, 8130, 8131, 8133, 8135, 8137, 8139, 8145, 8147, 8148, 8149, 8150, 8152, 8153, 8154, 8155, 8156, 8158, 8161, 8164, 8166, 8167, 8171, 8177, 8179, 8180, 8182, 8183, 8184, 8186, 8192, 8195, 8197, 8198, 8199, 8201, 8202, 8204, 8207, 8210, 8213, 8218, 8221, 8223, 8225, 8227, 8229, 8231,

Detection: 8092, 8097, 8106, 8109, 8120, 8140, 8141, 8159, 8162, 8172, 8196, 8205, 8211, 8215, 8219, 8226,

SEQ ID NO:19

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right Primer: 8233, 8239, 8240, 8242, 8243, 8246, 8248, 8252, 8257, 8261, 8264, 8266, 8268, 8271, 8275, 8278, 8282, 8284, 8285, 8286, 8287, 8288, 8291, 8299, 8302, 8303,

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Detection: 8234, 8241, 8249, 8262, 8265, 8272, 8276, 8279, 8283, 8290, 8300, 8308, 8320, 8338, 8355, 8358, 8368, 8380,

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Detection: 8386, 8389, 8393, 8396, 8400, 8404, 8408, 8417, 8421, 8427, 8430, 8439, 8441, 8443, 8453, 8459, 8471, 8483, 8495, 8507, 8509, 8525, 8538, 8540, 8543, 8549, 8553, 8559, 8561, 8573, 8579, 8584, 8590, 8593, 8605, 8611, 8622, 8626, 8656, 8664, 8721, 8724, 8731,

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right Primer: 8739, 8742, 8745, 8748, 8749, 8752, 8757, 8758, 8761, 8764, 8771, 8773, 8778, 8780, 8791, 8797, 8800, 8802, 8805, 8808, 8810, 8814, 8816, 8818, 8819, 8821, 8824, 8827, 8828, 8831, 8833, 8838, 8840, 8841, 8755, 8843, 8844, 8848, 8850, 8852, 8854, 8855, 8863, 8864, 8865, 8867, 8868, 8869, 8870, 8871, 8872, 8873, 8874, 8876, 8877, 8881, 8882, 8883, 8885, 8887, 8888, 8889, 8890, 8892, 8894, 8896, 8897, 8899, 8902, 8785, 8903, 8904, 8905, 8906, 8907, 8908, 8911,

Detection: 8740, 8743, 8746, 8765, 8774, 8781, 8792, 8795, 8811, 8817, 8822, 8825, 8834, 8839, 8849, 8858, 8860, 8862, 8875, 8886, 8910,

SEQ ID NO: 21

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right Primer: 8914, 8916, 8918, 8921, 8925, 8927, 8928, 8930, 8932, 8933, 8937,
Detection: 8915, 8919, 8922, 8926,

SEQ ID NO: 32

left Primer: 8938, 8944, 8945, 8948, 8952, 8953, 8954, 8957, 8958, 8961, 8962, 8965, 8967, 8969, 8970, 8971, 8972, 8977,

116

right Primer: 8939, 8941, 8942, 8943, 8946, 8949, 8950, 8951, 8955, 8960, 8963, 8964, 8966, 8968, 8974, 8975, 8976, 8978, 8979,

Detection: 8940, 8947, 8956, 8959, 8973,

SEQ ID NO: 33

left Primer: 8980, 8984, 8988, 8989, 8998, 9001, 9002, 9005, 9007, 9008, 9012, 9013, 9016, 9019, 9020, 9022, 9023, 9025, 9028, 9029, 9032, 9033, 9034, 9035, 9037, 9038, 9044, 9046, 9047, 9048, 9049, 9054,

right Primer: 8981, 8983, 8985, 8987, 8990, 8992, 8993, 8994, 8995, 8997, 8999, 9003, 9004, 9006, 9009, 9010, 9011, 9014, 9017, 9018, 9021, 9024, 9026, 9030, 9036, 9039, 9041, 9042, 9043, 9045, 9050, 9051, 9052, 9053,

Detection: 8982, 8986, 8991, 8996, 9000, 9015, 9027, 9031, 9040,

SEQ ID NO: 34

left Primer: 9055, 9058, 9060, 9063, 9066, 9067, 9068, 9069, 9070, 9071, 9074, 9075, 9077, 9078, 9079, 9080, 9082, 9084, 9088, 9089, 9090, 9099, 9103, 9104, 9105, 9107, 9111,

right Primer: 9056, 9061, 9064, 9072, 9081, 9083, 9085, 9086, 9087, 9091, 9092, 9093, 9094, 9095, 9096, 9097, 9098, 9100, 9102, 9106, 9108, 9109, 9110, 9112,

Detection: 9057, 9059, 9062, 9065, 9073, 9076, 9101,

SEQ ID NO: 24

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right Primer: 9114, 9119, 9121, 9122, 9123, 9124, 9127, 9131, 9133, 9134, 9135, 9137, 9138, 9139, 9140, 9142, 9144, 9146, 9147, 9149, 9151, 9152, 9154, 9155, 9156, 9160,

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Detection: 9115, 9117, 9120, 9132, 9145, 9161, 9170, 9179, 9196, 9210, 9213, 9225,

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SEQ ID NO: 35

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right Primer: 9293, 9296, 9298, 9301, 9305, 9309, 9312, 9314, 9316, 9317, 9318,

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9521, 9525, 9527, 9529, 9530, 9536, 9539, 9544, 9547, 9549, 9550,

Detection: 9294, 9299, 9306, 9315, 9330, 9341, 9343, 9346, 9350, 9359, 9362, 9366,

9369, 9373, 9381, 9387, 9392, 9396, 9404, 9427, 9442, 9463, 9472, 9476, 9481, 9484, 9486,

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SEQ ID NO: 25

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right Primer: 9552, 9554, 9558, 9561, 9567, 9571, 9574, 9576, 9581, 9583, 9584, 9589, 9592, 9594, 9595,

Detection: 9553, 9562, 9568, 9572, 9575, 9582, 9590, 9597,

SEQ ID NO: 36

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right Primer: 9599, 9601, 9603, 9606, 9607, 9609, 9611, 9615, 9619, 9623, 9624, 9628, 9630, 9631, 9634, 9635, 9637, 9639, 9641, 9647, 9649, 9650, 9652, 9653, 9659, 9660, 9663, 9664, 9670, 9671, 9679, 9682, 9684, 9687, 9690, 9692, 9693, 9695, 9697, 9700, 9701, 9703, 9706, 9708, 9712, 9713, 9714, 9716, 9717, 9718, 9721, 9724, 9727, 9730, 9734, 9739, 9742, 9751, 9753, 9757, 9760, 9761, 9762, 9763, 9764, 9765, 9766, 9767, 9769, 9773, 9775, 9778, 9780, 9783, 9786, 9788, 9791, 9793, 9794, 9795, 9798, 9801, 9803, 9804, 9807, 9808, 9809, 9810, 9811, 9813, 9816, 9817, 9819, 9820, 9822, 9826, 9828, 9830, 9833, 9836, 9838, 9840, 9843, 9846, 9847, 9848, 9849, 9850, 9852, 9856, 9858, 9859, 9861, 9864, 9866, 9867, 9868, 9869, 9870, 9872, 9876, 9877, 9880, 9881,

Detection: 9600, 9610, 9616, 9620, 9629, 9636, 9642, 9648, 9655, 9669, 9674, 9678, 9681, 9685, 9689, 9696, 9704, 9709, 9725, 9728, 9731, 9736, 9740, 9743, 9752, 9770, 9774, 9779, 9785, 9799, 9802, 9814, 9824, 9829, 9834, 9874,

SEQ ID NO: 28

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right Primer: 9883, 9885, 9887, 9889, 9892, 9893, 9895, 9896, 9897, 9898, 9899, 9901, 9903, 9905, 9907, 9909, 9913, 9914, 9916, 9918, 9920, 9921, 9922, 9924, 9926, 9929, 9931, 9932, 9933, 9934, 9937, 9938, 9939, 9941, 9942, 9944, 9945, 9946, 9947, 9948, 9950, 9952, 9955, 9959, 9960, 9961, 9966, 9967, 9970, 9972, 9973, 9974, 9976, 9979, 9980, 9982, 9983, 9984, 9985, 9986, 9987, 9993, 9994, 9996, 9998, 9999, 10000, 10001, 10002, 10003, 10004, 10005, 10006, 10007, 10008, 10009, 10010, 10013, 10015, 10016, 10019, 10020, 10021, 10022, 10023, 10025, 10026, 10027, 10028, 10030, 10036, 10038, 10040, 10043, 10044, 10045, 10047, 10048, 10049, 10051, 10053, 10055, 10056, 10057, 10060,

Detection: 9884, 9888, 9906, 9910, 9927, 9930, 9951, 9965, 9971, 9977, 10024, 10029, 10039, 10052,

SEQ ID NO: 37

left Primer: 10061, 10064, 10067, 10068, 10070, 10071, 10072, 10074, 10079, 10081, 10083, 10088, 10091, 10095, 10096, 10097, 10101, 10103, 10105, 10106, 10109, 10110, 10111, 10113, 10116, 10118, 10120, 10122, 10125, 10126, 10127, 10131, 10134, 10137, 10138, 10144, 10147, 10149, 10151, 10152, 10155, 10156, 10159, 10160, 10169, 10172, 10174, 10175, 10178, 10181, 10186, 10188, 10189, 10190, 10191, 10192, 10196, 10199, 10203, 10204, 10210, 10211, 10216, 10218, 10221, 10224, 10225, 10226, 10229, 10230, 10231, 10232, 10234, 10239, 10241, 10244, 10252, 10253, 10257, 10258, 10259, 10261, 10266, 10272, 10276,

right Primer: 10062, 10065, 10069, 10075, 10077, 10078, 10080, 10082, 10084, 10086, 10087, 10089, 10090, 10092, 10093, 10094, 10098, 10099, 10100, 10102, 10104, 10107, 10112, 10114, 10117, 10119, 10121, 10123, 10124, 10129, 10130, 10132, 10133,

10135, 10139, 10140, 10141, 10142, 10143, 10145, 10148, 10150, 10153, 10154, 10157,

10161, 10163, 10164, 10165, 10166, 10167, 10170, 10173, 10176, 10177, 10179, 10182,

10183, 10184, 10187, 10193, 10194, 10195, 10197, 10198, 10200, 10201, 10202, 10205,

10206, 10207, 10209, 10212, 10213, 10214, 10215, 10219, 10220, 10222, 10223, 10227,

10228, 10233, 10235, 10236, 10237, 10240, 10242, 10243, 10245, 10247, 10248, 10249,

10251, 10254, 10255, 10256, 10260, 10262, 10263, 10264, 10265, 10267, 10268, 10269,

10270, 10271, 10273, 10274, 10275, 10277, 10278,

Detection: 10063, 10066, 10073, 10076, 10085, 10108, 10115, 10128, 10136, 10146,

10158, 10162, 10168, 10171, 10180, 10185, 10208, 10217, 10238, 10246, 10250,

SEQ ID NO: 26

left Primer: 10279, 10282, 10285, 10287, 10291, 10292, 10297, 10299, 10301, 10304,

10306, 10308, 10310, 10314, 10317, 10318, 10320, 10322, 10327, 10329, 10330, 10333,

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10362, 10366, 10369, 10372, 10373, 10374, 10376, 10378, 10383, 10385, 10391, 10398,

10403, 10407, 10410, 10413, 10415, 10419, 10422, 10425, 10427, 10428, 10429, 10430,

10432, 10433, 10434, 10437, 10438, 10439, 10442, 10443, 10445, 10446, 10447, 10448,

10452, 10453, 10454, 10455, 10456, 10460, 10463, 10466, 10467, 10469, 10470, 10473,

10475, 10477, 10480, 10484, 10488, 10490, 10494, 10495, 10497, 10501, 10502, 10503,

10507, 10508, 10510, 10517, 10518, 10520, 10522, 10524, 10525, 10528, 10529, 10533,

10536, 10537, 10540, 10541, 10545, 10547, 10551, 10553, 10556, 10559, 10560, 10563,

10564, 10565, 10568, 10571, 10574, 10576, 10579, 10581, 10583, 10588, 10589, 10590,

10592, 10593, 10596, 10597, 10601, 10602, 10603, 10605, 10607, 10609, 10610, 10612,

10613, 10617, 10618, 10621, 10624, 10627, 10630, 10632, 10634, 10637, 10638, 10639,

10640, 10641, 10643, 10645, 10647, 10653, 10655, 10657, 10659, 10661, 10662, 10663,

10664, 10665, 10666, 10667, 10668, 10669, 10672, 10673, 10674, 10675,

right Primer: 10280, 10283, 10286, 10288, 10290, 10293, 10295, 10298, 10300,

10302, 10305, 10307, 10309, 10311, 10312, 10313, 10315, 10319, 10321, 10323, 10325,

10326, 10328, 10331, 10338, 10341, 10343, 10344, 10345, 10346, 10347, 10349, 10352,

10355, 10356, 10357, 10361, 10363, 10365, 10367, 10370, 10375, 10377, 10379, 10380, 10381, 10382, 10384, 10386, 10387, 10388, 10390, 10392, 10394, 10395, 10399, 10401, 10402, 10404, 10406, 10408, 10411, 10412, 10414, 10416, 10417, 10418, 10420, 10423, 10426, 10431, 10435, 10440, 10444, 10449, 10451, 10457, 10459, 10461, 10462, 10464, 10465, 10468, 10471, 10476, 10478, 10481, 10483, 10485, 10487, 10489, 10491, 10492, 10493, 10498, 10499, 10500, 10504, 10506, 10509, 10511, 10513, 10514, 10515, 10516, 10519, 10521, 10523, 10526, 10530, 10531, 10532, 10534, 10535, 10538, 10539, 10542, 10543, 10544, 10546, 10548, 10550, 10552, 10554, 10557, 10558, 10561, 10566, 10569, 10572, 10573, 10577, 10578, 10580, 10582, 10584, 10586, 10594, 10598, 10600, 10604, 10606, 10611, 10614, 10616, 10619, 10622, 10625, 10626, 10628, 10631, 10633, 10635, 10642, 10644, 10646, 10648, 10649, 10650, 10651, 10652, 10654, 10656, 10658, 10660, 10670, 10671, 10676,

Detection: 10281, 10284, 10289, 10294, 10296, 10303, 10316, 10324, 10332, 10334, 10339, 10353, 10364, 10368, 10371, 10389, 10393, 10396, 10397, 10400, 10405, 10409, 10421, 10424, 10436, 10441, 10450, 10458, 10472, 10474, 10479, 10482, 10486, 10496, 10505, 10512, 10527, 10549, 10555, 10562, 10567, 10570, 10575, 10585, 10587, 10591, 10595, 10599, 10608, 10615, 10620, 10623, 10629, 10636, 10677,

SEQ ID NO: 38

left Primer: 10678, 10684,

right Primer: 10679, 10681, 10682, 10683, 10685, 10687, 10688, 10689,

Detection: 10680, 10686,

SEQ ID NO: 39

left Primer: 10690,

right Primer: 10691,

Detection: 10692,

SEQ ID NO: 27

left Primer: 10693, 10696, 10699, 10703, 10704, 10709, 10711, 10712, 10713, 10720, 10721, 10722, 10724, 10725, 10726, 10728, 10729, 10734, 10740, 10742, 10743, 10744, 10745, 10746, 10748, 10749, 10751, 10754, 10758, 10759, 10760, 10764, 10768, 10769, 10772, 10775, 10780, 10781, 10783, 10786, 10791, 10796, 10801, 10808, 10817, 10818, 10819, 10821, 10823, 10826, 10827, 10829, 10830, 10834, 10843, 10844, 10847, 10848, 10849, 10853, 10856, 10858, 10866, 10869, 10872, 10874, 10877, 10878, 10879, 10882, 10884, 10885, 10889, 10892, 10893, 10896, 10900, 10901, 10903, 10905, 10907, 10909, 10910, 10911, 10914, 10916, 10919, 10926, 10930, 10932, 10933, 10934, 10938, 10941, 10942, 10943, 10944, 10946, 10947, 10953, 10955, 10956, 10958, 10959, 10962, 10967, 10968, 10972, 10975, 10977, 10978, 10981, 10984, 10985, 10988, 10992, 10995, 10997, 10999, 11000, 11001, 11006, 11009, 11013, 11016, 11020, 11025, 11033, 11036, 11040, 11041, 11042, 11043, 11045, 11046, 11048, 11052, 11055, 11060, 11061, 11062, 11064, 11065, 11067, 11070, 11071, 11072, 11078, 11079, 11081, 11085, 11093,

right Primer: 10694, 10697, 10700, 10702, 10705, 10707, 10710, 10714, 10716, 10717, 10718, 10719, 10723, 10727, 10730, 10732, 10733, 10735, 10737, 10738, 10739, 10741, 10747, 10750, 10752, 10755, 10757, 10761, 10762, 10763, 10765, 10767, 10770, 10773, 10776, 10778, 10779, 10782, 10784, 10788, 10789, 10790, 10792, 10793, 10794, 10798, 10799, 10800, 10802, 10804, 10805, 10806, 10807, 10809, 10811, 10812, 10813, 10814, 10815, 10816, 10820, 10822, 10824, 10831, 10833, 10835, 10837, 10838, 10839, 10840, 10841, 10842, 10845, 10846, 10850, 10851, 10854, 10857, 10859, 10860, 10861, 10862, 10863, 10864, 10865, 10868, 10870, 10871, 10873, 10875, 10880, 10881, 10883, 10886, 10888, 10890, 10894, 10897, 10899, 10902, 10904, 10906, 10908, 10912, 10915, 10917, 10920, 10922, 10924, 10925, 10927, 10928, 10929, 10931, 10935, 10937, 10939, 10945, 10948, 10950, 10951, 10952, 10954, 10957, 10960, 10963, 10965, 10966, 10969, 10970, 10971, 10973, 10976, 10979, 10980, 10982, 10986, 10987, 10989, 10991, 10993, 10996, 10998, 11002, 11004, 11005, 11007, 11010, 11012, 11014, 11015, 11017, 11019, 11021, 11022, 11023, 11026, 11027, 11028, 11029, 11030, 11031, 11032, 11034, 11037, 11039, 11044, 11047, 11049, 11050, 11051, 11053, 11054, 11056, 11058, 11059, 11063,

11066, 11068, 11069, 11073, 11074, 11075, 11076, 11077, 11080, 11082, 11083, 11084, 11086, 11087, 11088, 11089, 11090, 11091, 11092, 11094,

Detection: 10695, 10698, 10701, 10706, 10708, 10715, 10731, 10736, 10753, 10756, 10766, 10771, 10774, 10777, 10785, 10787, 10795, 10797, 10803, 10810, 10825, 10828, 10832, 10836, 10852, 10855, 10867, 10876, 10887, 10891, 10895, 10898, 10913, 10918, 10921, 10923, 10936, 10940, 10949, 10961, 10964, 10974, 10983, 10990, 10994, 11003, 11008, 11011, 11018, 11024, 11035, 11038, 11057, 11095,

SEQ ID NO: 40

left Primer: 11376, 11381, 11382, 11383, 11384, 11387, 11397, 11401, 11405, 11408, 11414, 11418, 11421, 11422, 11424, 11427, 11429, 11430,

right Primer: 11377, 11379, 11380, 11385, 11386, 11388, 11390, 11391, 11392, 11394, 11395, 11396, 11398, 11399, 11400, 11402, 11403, 11404, 11406, 11409, 11410, 11411, 11412, 11415, 11416, 11417, 11419, 11423, 11425, 11428, 11431, 11432, 11433,

Detection: 11378, 11389, 11393, 11407, 11413, 11420, 11426,

Light cycler dual probe assays:

SEQ ID NO: 41

left Primer: 11434, 11438, 11442, 11443, 11447, 11449, 11450, 11452, 11456, 11459, 11463, 11464, 11467, 11468, 11472, 11477, 11480, 11481, 11482, 11483, 11484, 11488, 11489, 11493, 11498, 11502, 11504, 11505, 11506, 11507, 11508, 11509, 11512, 11513, 11514, 11517, 11520, 11522, 11523, 11524, 11526, 11528, 11532, 11534,

right Primer: 11435, 11439, 11444, 11445, 11446, 11448, 11451, 11453, 11457, 11458, 11460, 11461, 11462, 11465, 11466, 11469, 11473, 11474, 11475, 11476, 11478, 11479, 11485, 11486, 11487, 11490, 11494, 11497, 11499, 11500, 11501, 11503, 11510, 11511, 11515, 11518, 11519, 11521, 11525, 11527, 11529,

Detection: 11436, 11440, 11454, 11470, 11491, 11495, 11530,

Anchor: 11437, 11441, 11455, 11471, 11492, 11496, 11516, 11531, 11533,

SEQ ID NO: 5

left Primer: 11535, 11539, 11540, 11541, 11548, 11550, 11551, 11552, 11555, 11556, 11557, 11560, 11561, 11562, 11568, 11569, 11571, 11572, 11573, 11577,

right Primer: 11536, 11542, 11545, 11546, 11547, 11549, 11553, 11554, 11563, 11566, 11570, 11574,

Detection: 11537, 11543, 11558, 11564, 11575,

Anchor: 11538, 11544, 11559, 11565, 11567, 11576,

SEQ ID NO: 6

left Primer: 11578, 11585, 11586, 11590, 11591, 11592, 11595, 11596, 11597, 11600,

right Primer: 11579, 11582, 11587, 11593, 11594, 11598, 11601,

Detection: 11580, 11583, 11588,

Anchor: 11581, 11584, 11589, 11599,

SEQ ID NO: 8

left Primer: 11602, 11606, 11607, 11608, 11609, 11616, 11618, 11622, 11626, 11628, 11632, 11633,

right Primer: 11603, 11610, 11613, 11614, 11615, 11617, 11619, 11623, 11627, 11629, 11634,

Detection: 11604, 11611, 11620, 11624, 11630,

Anchor: 11605, 11612, 11621, 11625, 11631,

SEQ ID NO: 16

left Primer: 11635, 11640, 11641, 11642, 11643, 11647, 11648, 11654, 11655, 11656, 11657, 11658,

right Primer: 11636, 11639, 11644, 11645, 11646, 11649, 11652, 11653,

Detection: 11637, 11650,

Anchor: 11638, 11651,

132

SEQ ID NO: 42

left Primer: 11659, 11663, 11664, 11665, 11666, 11670, 11671, 11672, 11673, 11677, 11681, 11687, 11690, 11694, 11696, 11697, 11701, 11705, 11709, 11713, 11719, 11723, 11729, 11733, 11739, 11744, 11745, 11748, 11749, 11751, 11755, 11759, 11763, 11770, 11772, 11774, 11776, 11778, 11782, 11785,

right Primer: 11660, 11667, 11674, 11675, 11676, 11678, 11682, 11685, 11686, 11688, 11689, 11691, 11692, 11693, 11695, 11698, 11699, 11700, 11702, 11706, 11710, 11716, 11720, 11724, 11725, 11727, 11728, 11730, 11731, 11732, 11734, 11735, 11736, 11737, 11738, 11740, 11743, 11746, 11747, 11750, 11752, 11756, 11761, 11762, 11764, 11765, 11767, 11768, 11769, 11773, 11775, 11777, 11779, 11783, 11784,

Detection: 11661, 11668, 11679, 11683, 11703, 11707, 11711, 11714, 11717, 11721, 11726, 11741, 11753, 11757, 11766, 11771, 11780,

Anchor: 11662, 11669, 11680, 11684, 11704, 11708, 11712, 11715, 11718, 11722, 11742, 11754, 11758, 11760, 11781,

SEQ ID NO: 14

left Primer: 11786, 11790, 11791, 11792,

right Primer: 11787, 11793,

Detection: 11788, 11794,

Anchor: 11789, 11795,

SEQ ID NO: 43

left Primer: 11796, 11800, 11802, 11803, 11805, 11810, 11818, 11819, 11823, 11824, 11827, 11828, 11829, 11833, 11836, 11837, 11839, 11840, 11847, 11850, 11852, 11857, 11860, 11864, 11866,

right Primer: 11797, 11801, 11804, 11806, 11809, 11811, 11814, 11815, 11817, 11820, 11825, 11830, 11835, 11841, 11844, 11845, 11846, 11848, 11851, 11853, 11856, 11858, 11861, 11862, 11863, 11865,

Detection: 11798, 11807, 11812, 11816, 11821, 11831, 11842, 11854,

Anchor: 11799, 11808, 11813, 11822, 11826, 11832, 11834, 11838, 11843, 11849, 11855, 11859,

SEQ ID NO: 15

left Primer: 11867, 11871, 11876, 11878, 11882, 11888, 11891, 11894,

right Primer: 11868, 11872, 11875, 11877, 11879, 11883, 11886, 11887, 11889, 11890, 11892, 11893, 11895,

Detection: 11869, 11873, 11880, 11884, 11896,

Anchor: 11870, 11874, 11881, 11885, 11897,

SEQ ID NO:4

left Primer: 11898, 11902, 11903, 11904, 11908, 11909, 11911, 11912, 11913, 11914, 11915, 11917, 11918, 11922, 11926, 11927, 11930, 11932, 11933, 11937, 11941, 11942, 11946, 11947, 11948, 11949, 11950, 11951, 11952, 11955, 11959, 11968, 11972, 11976, 11978, 11979, 11980, 11981, 11986, 11988, 11989, 11991, 11992, 11995, 11997, 11998, 12001, 12002, 12004, 12005,

right Primer: 11899, 11905, 11910, 11916, 11919, 11923, 11924, 11925, 11928, 11929, 11931, 11934, 11938, 11943, 11953, 11954, 11956, 11960, 11963, 11964, 11967, 11969, 11973, 11977, 11982, 11985, 11987, 11990, 11993, 11994, 11996, 11999, 12000, 12003,

Detection: 11900, 11906, 11920, 11935, 11939, 11944, 11957, 11961, 11965, 11970, 11974, 11983,

Anchor: 11901, 11907, 11921, 11936, 11940, 11945, 11958, 11962, 11966, 11971, 11975, 11984,

SEQ ID NO: 7

left Primer: 12006, 12011, 12015, 12016, 12017, 12021, 12022, 12023, 12024, 12025, 12026, 12027, 12031, 12034, 12042, 12043, 12045, 12049, 12050, 12051, 12054, 12062, 12063, 12064, 12066, 12067, 12068, 12070, 12072, 12076, 12082, 12085, 12086, 12089,

12090, 12093, 12094, 12095, 12096, 12097, 12098, 12101, 12103, 12107, 12108, 12111, 12112, 12114, 12118, 12119, 12120, 12128,

right Primer: 12007, 12010, 12012, 12020, 12028, 12032, 12033, 12035, 12038, 12039, 12044, 12046, 12052, 12055, 12056, 12057, 12058, 12059, 12060, 12061, 12065, 12069, 12071, 12073, 12077, 12080, 12081, 12084, 12087, 12088, 12091, 12099, 12100, 12102, 12104, 12109, 12110, 12113, 12115, 12121, 12122, 12123, 12124, 12125, 12127, 12129,

Detection: 12008, 12013, 12018, 12029, 12036, 12040, 12047, 12074, 12078, 12083, 12105, 12116,

Anchor: 12009, 12014, 12019, 12030, 12037, 12041, 12048, 12053, 12075, 12079, 12036, 12092, 12106, 12117, 12126,

SEQ ID NO:1

left Primer: 12130, 12135, 12136, 12141, 12144, 12147, 12148, 12149, 12153, 12159, 12160,

right Primer: 12131, 12134, 12137, 12138, 12139, 12140, 12142, 12143, 12145, 12146, 12150, 12154, 12157, 12158, 12161, 12162, 12163,

Detection: 12132, 12151, 12155,

Anchor: 12133, 12152, 12156,

SEQ ID NO: 45

left Primer: 12164, 12168, 12171, 12176, 12181, 12187, 12190, 12194, 12196, 12197, 12199, 12201, 12202, 12209, 12213, 12216, 12220, 12221, 12223, 12225, 12226, 12231, 12233, 12239, 12243, 12244, 12250, 12254, 12256, 12257, 12259, 12266, 12270, 12271, 12272, 12274, 12276, 12277, 12278, 12280, 12284, 12285, 12288, 12289, 12290, 12291, 12295, 12296, 12302, 12308, 12313, 12317, 12321, 12322, 12323, 12324, 12325, 12327, 12328, 12330, 12331, 12332, 12336, 12337, 12339, 12343, 12346, 12349, 12353, 12354, 12355, 12359, 12361, 12363, 12367, 12369, 12370, 12372, 12376, 12377, 12379, 12380, 12381, 12382, 12383, 12385, 12389, 12393, 12398, 12400, 12402, 12403, 12406, 12408,

12409, 12410, 12412, 12415, 12417, 12419, 12422, 12424, 12429, 12430, 12432, 12433, 12435, 12436, 12437, 12441, 12445, 12448, 12449, 12454, 12458, 12459, 12460,

right Primer: 12165, 12169, 12170, 12172, 12175, 12177, 12180, 12182, 12185, 12186, 12188, 12191, 12195, 12198, 12200, 12203, 12206, 12207, 12208, 12210, 12214, 12217, 12222, 12224, 12227, 12228, 12229, 12230, 12232, 12234, 12237, 12238, 12240, 12241, 12242, 12245, 12248, 12249, 12251, 12255, 12258, 12260, 12262, 12263, 12264, 12265, 12267, 12268, 12269, 12273, 12279, 12281, 12286, 12287, 12292, 12297, 12300, 12303, 12306, 12307, 12309, 12312, 12314, 12318, 12326, 12333, 12338, 12340, 12341, 12342, 12345, 12347, 12348, 12352, 12356, 12360, 12362, 12364, 12371, 12373, 12378, 12386, 12390, 12394, 12397, 12401, 12404, 12405, 12411, 12413, 12418, 12420, 12423, 12425, 12428, 12438, 12439, 12440, 12442, 12443, 12446, 12450, 12453, 12455,

Detection: 12166, 12173, 12178, 12183, 12189, 12192, 12204, 12211, 12218, 12235, 12246, 12252, 12282, 12293, 12298, 12301, 12304, 12310, 12315, 12319, 12334, 12350, 12357, 12365, 12374, 12387, 12391, 12395, 12416, 12426, 12444, 12451, 12456,

Anchor: 12167, 12174, 12179, 12184, 12193, 12205, 12212, 12215, 12219, 12236, 12247, 12253, 12261, 12275, 12283, 12294, 12299, 12305, 12311, 12316, 12320, 12329, 12335, 12344, 12351, 12358, 12366, 12368, 12375, 12384, 12388, 12392, 12396, 12399, 12407, 12414, 12421, 12427, 12431, 12434, 12447, 12452, 12457,

SEQ ID NO: 9

left Primer: 12461, 12465, 12467, 12468, 12472, 12474, 12477, 12480, 12484, 12486, 12488, 12489, 12490, 12493, 12495, 12499, 12501, 12504, 12506,

right Primer: 12462, 12469, 12475, 12478, 12481, 12483, 12485, 12487, 12492, 12494, 12496, 12497, 12498, 12500, 12502, 12505,

Detection: 12463, 12470, 12473, 12482,

Anchor: 12464, 12466, 12471, 12476, 12479, 12491, 12503,

SEQ ID NO: 46

left Primer: 12507, 12511, 12517, 12520, 12522, 12523, 12524, 12528, 12530, 12534, 12535, 12536, 12537, 12541, 12542, 12547, 12550, 12551, 12552, 12554, 12555, 12560, 12564, 12565, 12569, 12571, 12574, 12579, 12581, 12586, 12590, 12592, 12593, 12594, 12596, 12600, 12601, 12602, 12604, 12610, 12613, 12614, 12615, 12616, 12620, 12626, 12630,

right Primer: 12508, 12512, 12515, 12518, 12521, 12525, 12529, 12531, 12538, 12543, 12546, 12548, 12549, 12553, 12556, 12559, 12561, 12566, 12570, 12572, 12573, 12575, 12578, 12582, 12585, 12587, 12595, 12597, 12599, 12603, 12605, 12608, 12609, 12611, 12617, 12618, 12621, 12624, 12625, 12627,

Detection: 12509, 12513, 12516, 12514, 12532, 12539, 12544, 12557, 12562, 12567, 12576, 12583, 12588, 12591, 12606, 12612, 12622, 12628,

Anchor: 12510, 12514, 12519, 12526, 12527, 12533, 12540, 12545, 12558, 12563, 12568, 12577, 12580, 12584, 12589, 12598, 12607, 12619, 12623, 12629,

SEQ ID NO: 10

left Primer: 12631, 12636, 12637, 12638, 12639, 12643, 12647, 12648, 12652, 12654, 12660, 12664, 12666, 12670, 12672, 12675, 12677, 12680, 12686, 12691, 12692, 12695, 12699, 12700, 12701, 12703, 12705, 12709, 12710, 12714, 12715, 12716, 12718, 12719, 12720, 12724, 12725, 12728, 12732, 12738, 12739, 12743, 12744, 12745, 12747, 12748, 12752, 12757, 12763, 12764, 12766, 12767, 12768, 12771, 12776, 12777, 12781, 12785, 12787, 12788, 12791, 12792, 12796, 12797, 12800, 12804, 12808, 12813, 12815, 12816, 12817, 12822, 12825, 12828, 12831, 12833, 12834, 12837, 12839, 12841, 12842,

right Primer: 12632, 12635, 12640, 12641, 12644, 12649, 12653, 12655, 12658, 12661, 12662, 12663, 12665, 12667, 12671, 12673, 12678, 12679, 12681, 12682, 12683, 12684, 12685, 12687, 12688, 12689, 12690, 12693, 12694, 12696, 12702, 12704, 12706, 12712, 12713, 12717, 12721, 12726, 12729, 12730, 12731, 12733, 12736, 12737, 12740, 12746, 12749, 12750, 12751, 12753, 12754, 12755, 12756, 12758, 12760, 12761, 12762, 12765, 12769, 12770, 12772, 12775, 12778, 12782, 12786, 12789, 12790, 12793, 12794,

12795, 12798, 12799, 12801, 12805, 12809, 12812, 12814, 12818, 12821, 12823, 12824, 12826, 12827, 12832, 12835, 12836, 12838, 12840, 12843, 12844,

Detection: 12633, 12642, 12645, 12650, 12656, 12668, 12674, 12676, 12697, 12707, 12722, 12734, 12741, 12759, 12773, 12779, 12783, 12802, 12806, 12810, 12819, 12829,

Anchor: 12634, 12646, 12651, 12657, 12659, 12669, 12698, 12708, 12711, 12723, 12707, 12727, 12735, 12742, 12774, 12780, 12784, 12803, 12807, 12811, 12820, 12830,

SEQ ID NO: 11

left Primer: 12845, 12849, 12850, 12854, 12857, 12862, 12866, 12867, 12868, 12870, 12872, 12880, 12881, 12884, 12885, 12889, 12890, 12892, 12896, 12898, 12899, 12903, 12907, 12910, 12913, 12919, 12920, 12921, 12923, 12928, 12932, 12938, 12942, 12945, 12948, 12950, 12951, 12953, 12959,

right Primer: 12846, 12851, 12855, 12858, 12861, 12863, 12869, 12871, 12873, 12876, 12877, 12878, 12879, 12882, 12883, 12886, 12887, 12888, 12891, 12893, 12894, 12895, 12900, 12901, 12902, 12904, 12908, 12909, 12911, 12912, 12914, 12917, 12918, 12924, 12927, 12929, 12933, 12936, 12937, 12939, 12943, 12949, 12954, 12957, 12958, 12960,

Detection: 12847, 12852, 12859, 12864, 12874, 12905, 12915, 12925, 12930, 12934, 12940, 12944, 12946, 12955,

Anchor: 12848, 12853, 12856, 12860, 12865, 12875, 12897, 12906, 12916, 12922, 12926, 12931, 12935, 12941, 12930, 12947, 12952, 12956,

SEQ ID NO: 12

left Primer: 12961, 12965, 12969, 12975, 12977,

right Primer: 12962, 12966, 12967, 12968, 12970, 12971, 12972, 12973, 12974, 12976, 12978, 12981,

Detection: 12963, 12979,

Anchor: 12964, 12980,

SEQ ID NO: 13

left Primer: 12982, 12986, 12989, 12991, 12992, 12993, 12995, 12999, 13001,

right Primer: 12983, 12987, 12996, 12997, 12998, 13000, 13002,

Detection: 12984, 13003,

Anchor: 12985, 12988, 12990, 12994, 13004,

SEQ ID NO:3

left Primer: 13005, 13009, 13013, 13017, 13019, 13025, 13029, 13030, 13032, 13033, 13034, 13037, 13040, 13042, 13043, 13044, 13048, 13049, 13051, 13052, 13053, 13057, 13062, 13064, 13065, 13066, 13067, 13072, 13073, 13076, 13078, 13082, 13083, 13085, 13087, 13088, 13089, 13092, 13096, 13100, 13101, 13102, 13105, 13108, 13109, 13110, 13114, 13117, 13118, 13124, 13129, 13131, 13132, 13138, 13144, 13146, 13147, 13148, 13152, 13156, 13159, 13164, 13167, 13168, 13170, 13171, 13172, 13173, 13174, 13184, 13185,

right Primer: 13006, 13010, 13014, 13018, 13020, 13021, 13022, 13023, 13024, 13026, 13031, 13035, 13036, 13038, 13039, 13041, 13045, 13050, 13054, 13058, 13061, 13063, 13068, 13069, 13070, 13071, 13074, 13075, 13077, 13079, 13084, 13086, 13090, 13091, 13093, 13097, 13104, 13107, 13111, 13115, 13119, 13121, 13122, 13123, 13125, 13126, 13127, 13128, 13130, 13133, 13137, 13139, 13142, 13143, 13145, 13149, 13153, 13157, 13158, 13160, 13163, 13165, 13166, 13169, 13175, 13176, 13177, 13178, 13179, 13180, 13181, 13182, 13183, 13186, 13189, 13190,

Detection: 13007, 13011, 13015, 13027, 13046, 13055, 13059, 13080, 13094, 13098, 13112, 13120, 13134, 13140, 13150, 13154, 13161, 13187,

Anchor: 13008, 13012, 13016, 13028, 13047, 13056, 13060, 13081, 13095, 13099, 13103, 13106, 13113, 13116, 13135, 13136, 13141, 13151, 13155, 13162, 13188,

SEQ ID NO: 47

left Primer: 13191, 13195, 13196, 13197, 13199, 13200, 13204, 13206,

right Primer: 13192, 13198, 13201,

Detection: 13193, 13202,

Anchor: 13194, 13203, 13205,

SEQ ID NO: 18

left Primer: 13207, 13212, 13213, 13226, 13228, 13231, 13235, 13240, 13245, 13247, 13248, 13250, 13254, 13256, 13258, 13264, 13265, 13268, 13269, 13273, 13274, 13277, 13279, 13280, 13281, 13286,

right Primer: 13208, 13211, 13214, 13217, 13220, 13222, 13224, 13225, 13227, 13229, 13232, 13233, 13234, 13237, 13239, 13241, 13242, 13244, 13246, 13249, 13251, 13255, 13257, 13259, 13262, 13263, 13266, 13267, 13270, 13275, 13276, 13282, 13285,

Detection: 13209, 13215, 13218, 13236, 13252, 13260, 13271, 13283,

Anchor: 13210, 13216, 13219, 13221, 13223, 13230, 13238, 13243, 13253, 13261, 13272, 13278, 13284,

SEQ ID NO: 32

left Primer: 13641, 13645, 13646, 13649, 13650, 13652, 13653, 13654, 13655, 13658, 13659,

right Primer: 13642, 13647, 13648, 13656,

Detection: 13643,

Anchor: 13644, 13651, 13657,

SEQ ID NO: 33

left Primer: 13660,

right Primer: 13661,

Detection: 13662,

Anchor: 13663,

SEQ ID NO: 34

left Primer: 13664, 13669, 13670, 13671, 13672, 13673, 13675, 13676, 13677, 13681, 13683, 13685, 13687, 13689, 13691, 13693, 13696,

right Primer: 13665, 13668, 13674, 13678, 13682, 13684, 13686, 13688, 13690, 13692, 13694, 13695,

Detection: 13666, 13679,

Anchor: 13667, 13680,

SEQ ID NO: 24

left Primer: 13697, 13701, 13702, 13703, 13709, 13713, 13714, 13715, 13719, 13721, 13722, 13726, 13728, 13732, 13735, 13740,

right Primer: 13698, 13704, 13705, 13706, 13707, 13708, 13710, 13716, 13717, 13718, 13720, 13723, 13724, 13725, 13727, 13729, 13733, 13734, 13736, 13739, 13741, 13744,

Detection: 13699, 13711, 13730, 13737, 13742,

Anchor: 13700, 13712, 13731, 13738, 13743,

SEQ ID NO: 35

left Primer: 13745, 13749, 13751, 13752, 13753, 13754, 13758, 13762, 13765, 13766, 13768, 13772, 13773, 13774, 13777, 13778, 13779, 13783, 13785, 13790, 13791, 13797, 13802, 13803, 13805, 13806, 13807, 13809, 13812, 13814, 13816, 13817, 13818, 13819, 13820, 13823, 13824, 13826, 13828,

right Primer: 13746, 13755, 13759, 13763, 13764, 13767, 13769, 13775, 13776, 13780, 13784, 13786, 13788, 13789, 13792, 13795, 13796, 13798, 13800, 13804, 13808, 13813, 13815, 13821, 13822, 13829,

Detection: 13747, 13756, 13760, 13781, 13793, 13801, 13810, 13830,

Anchor: 13748, 13750, 13757, 13761, 13770, 13771, 13782, 13787, 13794, 13799, 13811, 13825, 13827, 13831,

SEQ ID NO: 36

left Primer: 13832, 13837, 13841, 13844, 13845, 13846, 13849, 13854, 13855, 13856, 13857, 13861, 13865, 13869, 13871, 13875, 13879, 13880,

right Primer: 13833, 13836, 13838, 13843, 13847, 13850, 13853, 13858, 13862, 13866, 13870, 13872, 13876, 13881, 13882,

Detection: 13834, 13839, 13851, 13859, 13863, 13867, 13873, 13877,

Anchor: 13835, 13840, 13842, 13848, 13852, 13860, 13864, 13868, 13874, 13878,

SEQ ID NO: 28

left Primer: 13883, 13889, 13894, 13902, 13903, 13910, 13911, 13914, 13915, 13916, 13928, 13929, 13930, 13931, 13935, 13936, 13937, 13944, 13947, 13948, 13950, 13960, 13961, 13962, 13964, 13965, 13966, 13968, 13970, 13971, 13972, 13976, 13978,

right Primer: 13884, 13887, 13890, 13893, 13895, 13898, 13900, 13904, 13905, 13906, 13907, 13908, 13909, 13912, 13913, 13917, 13920, 13921, 13922, 13923, 13924, 13925, 13926, 13932, 13938, 13941, 13942, 13943, 13945, 13946, 13949, 13953, 13954, 13956, 13957, 13958, 13959, 13963, 13967, 13969, 13974, 13975, 13977, 13980,

Detection: 13885, 13886, 13891, 13896, 13901, 13918, 13927, 13933, 13939, 13951,

Anchor: 13886, 13888, 13892, 13897, 13899, 13901, 13919, 13934, 13940, 13952, 13955, 13973, 13979,

SEQ ID NO: 37

left Primer: 13981, 13988, 13989, 13990, 13992, 13994, 13995, 13999,

right Primer: 13982, 13985, 13986, 13987, 13991, 13993, 13996, 14000, 14001, 14002, 14003,

Detection: 13983, 13997,

Anchor: 13984, 13998,

SEQ ID NO: 26

left Primer: 14004, 14009, 14014, 14017, 14020, 14024, 14026, 14029, 14035, 14036, 14040, 14043, 14045, 14051, 14057, 14058, 14059, 14063, 14065, 14066, 14067, 14068,

14069, 14070, 14071, 14073, 14074, 14078, 14079, 14080, 14081, 14082, 14083, 14084, 14085, 14087, 14094, 14095, 14100, 14108, 14109, 14113, 14114, 14118, 14120, 14121, 14122, 14130, 14133, 14138, 14141, 14142, 14143, 14147,

right Primer: 14005, 14008, 14010, 14013, 14015, 14018, 14021, 14025, 14027, 14028, 14030, 14033, 14034, 14037, 14038, 14039, 14041, 14042, 14044, 14046, 14049, 14050, 14052, 14055, 14056, 14060, 14064, 14072, 14077, 14086, 14088, 14089, 14090, 14091, 14092, 14093, 14096, 14097, 14098, 14099, 14101, 14102, 14103, 14104, 14105, 14106, 14107, 14110, 14115, 14123, 14126, 14127, 14132, 14134, 14135, 14136, 14137, 14139, 14140, 14144, 14145, 14148,

Detection: 14006, 14011, 14019, 14022, 14031, 14047, 14053, 14061, 14075, 14111, 14116, 14124, 14149,

Anchor: 14007, 14012, 14016, 14023, 14032, 14048, 14054, 14062, 14076, 14112, 14117, 14119, 14125, 14128, 14129, 14131, 14146, 14150,

SEQ ID NO: 27

left Primer: 14151, 14155, 14157, 14161, 14166, 14171, 14175, 14183, 14187, 14190, 14192, 14194, 14195, 14196, 14200, 14201,

right Primer: 14152, 14156, 14158, 14162, 14165, 14167, 14170, 14172, 14176, 14179, 14180, 14182, 14184, 14185, 14186, 14188, 14189, 14191, 14193, 14197, 14198, 14199, 14202, 14203,

Detection: 14153, 14159, 14163, 14168, 14173, 14177,

Anchor: 14154, 14160, 14164, 14169, 14174, 14178, 14181,

SEQ ID NO: 31

left Primer: 14204, 14210, 14211, 14216, 14217, 14223, 14226, 14228, 14229, 14230, 14233, 14235, 14239, 14241, 14243, 14244, 14250, 14254, 14255, 14256, 14257, 14260, 14261, 14262, 14267, 14268, 14269, 14273, 14274, 14277, 14281, 14284, 14286, 14289, 14292, 14293, 14297, 14299, 14301, 14304, 14305, 14306, 14312, 14316, 14320, 14322,

14324, 14328, 14329, 14331, 14337, 14342, 14344, 14346, 14347, 14349, 14350, 14353, 14356, 14361, 14363, 14364, 14365, 14366,

right Primer: 14205, 14208, 14212, 14215, 14218, 14221, 14224, 14225, 14227, 14231, 14236, 14240, 14242, 14245, 14246, 14247, 14251, 14263, 14266, 14270, 14278, 14282, 14283, 14285, 14287, 14290, 14291, 14294, 14298, 14300, 14302, 14303, 14307, 14310, 14313, 14317, 14323, 14325, 14327, 14330, 14334, 14338, 14340, 14341, 14345, 14348, 14351, 14352, 14354, 14355, 14357, 14358, 14359, 14360, 14367,

Detection: 14206, 14213, 14219, 14237, 14248, 14252, 14258, 14264, 14271, 14275, 14279, 14295, 14308, 14311, 14314, 14318, 14332, 14335, 14339, 14343,

Anchor: 14207, 14209, 14214, 14220, 14222, 14232, 14234, 14238, 14249, 14253, 14259, 14265, 14272, 14276, 14280, 14288, 14296, 14309, 14315, 14319, 14321, 14326, 14333, 14336, 14362,

SEQ ID NO: 40

left Primer: 14368, 14372, 14373, 14375, 14386,

right Primer: 14369, 14374, 14376, 14379, 14380, 14381, 14382, 14383, 14384, 14385,

Detection: 14370, 14377,

Anchor: 14371, 14378,

In an alternative most preferred embodiment of the method, the subsequent amplification of d) is carried out in the presence of *blocking oligonucleotides*, as described above. Said *blocking oligonucleotides* comprising a sequence having a length of at least 9 nucleotides which hybridizes to a pretreated nucleic acid sequence according to one of SEQ ID NOS:304 to SEQ ID NO:535 and sequences complementary thereto, wherein the base sequence of said oligomers comprises at least one CpG, TpG or CpA dinucleotide. Step e) of the method, namely the detection of the specific amplificates indicative of the methylation status of one or more CpG positions according to SEQ ID NOS:1 to SEQ ID NO:58 is carried out by means of real-time detection methods as described above.

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In a further preferred embodiment of the method the sequence(s) of the blocking oligonucleotides are selected from the group consisting of SEQ ID NOS:2114, 2119, 2124, 2125, 2127, 2132, 2133, 2135, 2137, 2138, 2139, 2140, 2141, 2142, 2143, 2144, 2146, 2147, 2148, 2149, 2150, 2151, 2152, 2153, 2154, 2155, 2156, 2157, 2158, 2159, 2160, 2031, 2082, 2087, 2089, 2094, 2095, 2096, 2097, 2099, 2107, 1980, 2018, 2022, 2023, 2026, 1939, 1966, 1973, 1974, 1975, 1926, 1930, 1931, 1932, 1933, 1934, 1903, 1917, 1921, 1834, 1896, 1805, 1819, 1821, 1823, 1824, 1825, 1826, 1827, 1828, 1829, 1770, 1782, 1785, 1786, 1788, 1789, 1790, 1792, 1795, 1796, 1797, 1798, 1799, 1800, 1748, 1759, 1760, 1761, 1762, 1763, 1764, 1765, 1724, 1741, 1597, 1609, 1610, 1611, 1612, 1613, 1614, 1615, 1616, 1617, 1618, 1619, 1620, 1621, 1622, 1623, 1624, 1625, 1626, 1627, 1628, 1629, 1630, 1631, 1633, 1634, 1635, 1636, 1637, 1638, 1639, 1640, 1641, 1642, 1643, 1644, 1645, 1646, 1647, 1648, 1649, 1650, 1651, 1652, 1653, 1654, 1655, 1656, 1657, 1658, 1659, 1660, 1661, 1662, 1663, 1668, 1684, 1685, 1686, 1687, 1688, 1689, 1690, 1691, 1692, 1693, 1694, 1695, 1696, 1697, 1698, 1699, 1700, 1701, 1706, 1712, 1713, 1715, 1716, 1717, 1718, 1719, 1571, 1579, 1581, 1584, 1585, 1586, 1587, 1588, 1591, 1592, 1537, 1555, 1556, 1557, 1558, 1559, 1560, 1561, 1562, 1563, 1564, 1565, 1566, 1452, 1455, 1456, 1457, 1458, 1463, 1514, 1515, 1516, 1517, 1518, 1519, 1524, 1525, 1526, 1527, 1528, 1529, 1531, 1532, 1372, 1423, 1424, 1425, 1427, 1432, 1433, 1434, 1435, 1436, 1437, 1440, 1441, 1442, 1443, 1444, 1445, 1446, 1447, 1343, 1358, 1359, 1360, 1361, 1362, 1363, 1364, 1365, 1366, 1367, 1310, 1315, 1316, 1317, 1318, 1319, 1320, 1321, 1322, 1324, 1325, 1327, 1328, 1330, 1332, 1334, 1337, 1338, 1276, 1290, 1305, 1203, 1228, 1238, 1239, 1240, 1247, 1248, 1255, 1256, 1265, 1268, 1269, 1164, 1171, 1172, 1173, 1174, 1176, 1177, 1178, 1179, 1180, 1181, 1182, 1183, 1184, 1185, 1186, 1187, 1188, 1189, 1190, 1191, 1192, 1193, 1194, 1195, 1196, 1197, 1198, 2725, 2748, 2749, 2750, 2710, 2717, 2718, 2719, 2720, 2721, 2684, 2699, 2703, 2704, 2705, 2706, 2658, 2662, 2679, 2603, 2613, 2617, 2585, 2594, 2595, 2596, 2597, 2598, 2599, 2489, 2497, 2498, 2499, 2500, 2501, 2504, 2505, 2507, 2508, 2510, 2511, 2512, 2513, 2514, 2515, 2519, 2520, 2521, 2522, 2525, 2526, 2527, 2528, 2529, 2530, 2531, 2532, 2534, 2535, 2536, 2537, 2539, 2540, 2541, 2542, 2543, 2544, 2545, 2546, 2547, 2550, 2551, 2552, 2553, 2554, 2558, 2563, 2564, 2572, 2580, 2581, 2472, 2482, 2484, 2408, 2464, 2465, 2466, 2467, 2468, 2339, 2349, 2367, 2370, 2372, 2373,

2374, 2375, 2376, 2377, 2378, 2379, 2380, 2382, 2386, 2387, 2389, 2393, 2394, 2395, 2396,

2397, 2398, 2402, 2403, 2404, 2327, 2333, 2334, 2335, 2305, 2315, 2318, 2260, 2268, 2270,

2271, 2272, 2273, 2274, 2275, 2276, 2277, 2278, 2279, 2280, 2281, 2282, 2283, 2284, 2285,

2286, 2287, 2288, 2289, 2290, 2291, 2292, 2293, 2294, 2295, 2296, 2297, 2298, 2299, 2300,

2301, 2232, 2245, 2246, 2248, 2249, 2250, 2251, 2252, 2254, 2255, 2256, 2210, 2218, 2219,

2221, 2222, 2223, 2227, 2228, 2202, 2204, 2205, 2206, 2164, 2180, 2183, 2187, 2188, 2189,

2190, 2191, 2192, 2193, 2194, 2195, 2196, 2197 and 2198.

Step e) of the method, namely the detection of the specific amplificates indicative of the methylation status of one or more CpG positions according to SEQ ID NOS:1 to SEQ ID NO:58 is carried out by means of real-time detection methods as described above, and wherein the sequence of said hybridization probes is selected from the group consisting of SEQ ID NOS:1162, 1163, 1201, 1202, 1208, 1211, 1217, 1274, 1288, 1275, 1289, 1299, 1308, 1309, 1341, 1342, 1347, 1351, 1354, 1372, 1423, 1424, 1425, 1427, 1432, 1433, 1434, 1435, 1436, 1437, 1440, 1441, 1442, 1443, 1444, 1445, 1446, 1447, 1370, 1371, 1400, 1450, 1461, 1451, 1462, 1467, 1470, 1473, 1475, 1535, 1536, 1542, 1544, 1569, 1570, 1583, 1590, 1595, 1666, 1704, 1596, 1601, 1603, 1667, 1705, 1722, 1723, 1727, 1735, 1746, 1747, 1768, 1769, 1773, 1803, 1804, 1807, 1810, 1832, 1833, 1836, 1867, 1871, 1901, 1902, 1908, 1911, 1924, 1925, 1937, 1938, 1943, 1946, 1949, 1956, 1957, 1978, 1979, 1986, 1992, 1998, 2001, 2003, 2010, 2020, 2025, 2029, 2030, 2113, 2163, 2201, 2209, 2231, 2259, 2304, 2326, 2338, 2348, 2351, 2369, 2407, 2471, 2488, 2557, 2584, 2602, 2616, 2657, 2661, 2664, 2671, 2683, 2709 and SEQ ID NO: 2724.

For each genomic sequence listed, the following oligonucleotides as detailed in the sequence listing may be used for a combined Blocking oligonucleotide-RealTime analysis: Lightcycler dual probe detection assays:

SEQ ID NO: 41

      left Primer: 1161, 1165, 1166, 1167,

      right Primer: 1160, 1168, 1169, 1170, 1175,

Blocker: 1164, 1171, 1172, 1173, 1174, 1176, 1177, 1178, 1179, 1180, 1181, 1182, 1183, 1184, 1185, 1186, 1187, 1188, 1189, 1190, 1191, 1192, 1193, 1194, 1195, 1196, 1197, 1198,

Detection: 1162,

Anchor: 1163,

SEQ ID NO: 6

left Primer: 1200, 1204, 1205, 1206, 1207, 1209, 1210, 1212, 1213, 1214, 1215, 1216, 1218,

right Primer: 1199, 1219, 1220, 1221, 1222, 1223, 1224, 1225, 1226, 1227, 1229, 1230, 1231, 1232, 1233, 1234, 1235, 1236, 1237, 1241, 1242, 1243, 1244, 1245, 1246, 1249, 1250, 1251, 1252, 1253, 1254, 1257, 1258, 1259, 1260, 1261, 1262, 1263, 1264, 1266, 1267, 1270, 1271,

Blocker: 1203, 1228, 1238, 1239, 1240, 1247, 1248, 1255, 1256, 1265, 1268, 1269,

Detection: 1201,

Anchor: 1202, 1208, 1211, 1217,

SEQ ID NO: 14

left Primer: 1273, 1277, 1278, 1279, 1280, 1281, 1282, 1283, 1287, 1291, 1292, 1293, 1294, 1295, 1296, 1297, 1298, 1300, 1301, 1302, 1303,

right Primer: 1272, 1284, 1285, 1286, 1304,

Blocker: 1276, 1290, 1305,

Detection: 1274, 1288,

Anchor: 1275, 1289, 1299,

SEQ ID NO: 7

left Primer: 1307, 1311,

right Primer: 1306, 1312, 1313, 1314, 1323, 1326, 1329, 1331, 1333, 1335, 1336,

Blocker: 1310, 1315, 1316, 1317, 1318, 1319, 1320, 1321, 1322, 1324, 1325, 1327, 1328, 1330, 1332, 1334, 1337, 1338,

Detection: 1308,

Anchor: 1309,

SEQ ID NO: 44

left Primer: 1340, 1344, 1345, 1346, 1348, 1349, 1350, 1352, 1353, 1355,

right Primer: 1339, 1356, 1357,

Blocker: 1343, 1358, 1359, 1360, 1361, 1362, 1363, 1364, 1365, 1366, 1367,

Detection: 1341,

Anchor: 1342, 1347, 1351, 1354,

SEQ ID NO:1

left Primer: 1369, 1373, 1374, 1375, 1376, 1377, 1378, 1379, 1380, 1381, 1382, 1383, 1384, 1385, 1386, 1387, 1388, 1389, 1390, 1391, 1392, 1393, 1394, 1395, 1396, 1397, 1398, 1399, 1401, 1402, 1403, 1404, 1405, 1406, 1407, 1408, 1409, 1410, 1411, 1412, 1413, 1414, 1415, 1416, 1417, 1418, 1419, 1420, 1421,

right Primer: 1368, 1422, 1426, 1428, 1429, 1430, 1431, 1438, 1439,

Blocker: 1372, 1423, 1424, 1425, 1427, 1432, 1433, 1434, 1435, 1436, 1437, 1440, 1441, 1442, 1443, 1444, 1445, 1446, 1447,

Detection: 1370,

Anchor: 1371, 1400,

SEQ ID NO: 9

left Primer: 1449, 1453, 1454, 1460, 1464, 1465, 1466, 1468, 1469, 1471, 1472, 1474, 1476, 1477, 1478, 1479, 1480, 1481, 1482, 1483, 1484, 1485, 1486, 1487, 1488, 1489, 1490, 1491, 1492, 1493, 1494, 1495, 1496, 1497, 1498, 1499, 1500, 1501, 1502, 1503, 1504, 1505, 1506, 1507, 1508, 1509, 1510,

right Primer: 1448, 1459, 1511, 1512, 1513, 1520, 1521, 1522, 1523, 1530,

Blocker: 1452, 1455, 1456, 1457, 1458, 1463, 1514, 1515, 1516, 1517, 1518, 1519, 1524, 1525, 1526, 1527, 1528, 1529, 1531, 1532,

Detection: 1450, 1461,

Anchor: 1451, 1462, 1467, 1470, 1473, 1475,

SEQ ID NO: 46

left Primer: 1534, 1538, 1539, 1540, 1541, 1543, 1545, 1546, 1547, 1548, 1549, 1550, 1551, 1552, 1553, 1554,

right Primer: 1533,

Blocker: 1537, 1555, 1556, 1557, 1558, 1559, 1560, 1561, 1562, 1563, 1564, 1565, 1566,

Detection: 1535,

Anchor: 1536, 1542, 1544,

SEQ ID NO: 10

left Primer: 1568, 1572, 1573,

right Primer: 1567, 1574, 1575, 1576, 1577, 1578, 1580, 1582, 1589,

Blocker: 1571, 1579, 1581, 1584, 1585, 1586, 1587, 1588, 1591, 1592,

Detection: 1569,

Anchor: 1570, 1583, 1590,

SEQ ID NO: 12

left Primer: 1594, 1598, 1599, 1600, 1602, 1604, 1605, 1606, 1607, 1665, 1669, 1670,
1671, 1672, 1673, 1674, 1675, 1676, 1677, 1678, 1679, 1680, 1681, 1682, 1703, 1707, 1708,

right Primer: 1593, 1608, 1632, 1664, 1683, 1702, 1709, 1710, 1711, 1714,

Blocker: 1597, 1609, 1610, 1611, 1612, 1613, 1614, 1615, 1616, 1617, 1618, 1619,
1620, 1621, 1622, 1623, 1624, 1625, 1626, 1627, 1628, 1629, 1630, 1631, 1633, 1634, 1635,
1636, 1637, 1638, 1639, 1640, 1641, 1642, 1643, 1644, 1645, 1646, 1647, 1648, 1649, 1650,
1651, 1652, 1653, 1654, 1655, 1656, 1657, 1658, 1659, 1660, 1661, 1662, 1663, 1668, 1684,
1685, 1686, 1687, 1688, 1689, 1690, 1691, 1692, 1693, 1694, 1695, 1696, 1697, 1698, 1699,
1700, 1701, 1706, 1712, 1713, 1715, 1716, 1717, 1718, 1719,

Detection: 1595, 1666, 1704,

Anchor: 1596, 1601, 1603, 1667, 1705,

SEQ ID NO: 13

left Primer: 1721, 1725, 1726, 1728, 1729, 1730, 1731, 1732, 1733, 1734, 1736, 1737,

right Primer: 1720, 1738, 1739, 1740, 1742, 1743,

Blocker: 1724, 1741,

Detection: 1722,

Anchor: 1723, 1727, 1735,

SEQ ID NO:3

left Primer: 1745, 1749, 1750, 1751, 1752, 1753, 1754, 1755, 1758,

right Primer: 1744, 1756, 1757,

Blocker: 1748, 1759, 1760, 1761, 1762, 1763, 1764, 1765,

Detection: 1746,

Anchor: 1747,

SEQ ID NO: 18

left Primer: 1767, 1771, 1772, 1774, 1775, 1776, 1777, 1778,

right Primer: 1766, 1779, 1780, 1781, 1783, 1784, 1787, 1791, 1793, 1794,

Blocker: 1770, 1782, 1785, 1786, 1788, 1789, 1790, 1792, 1795, 1796, 1797, 1798,
1799, 1800,

Detection: 1768,

Anchor: 1769, 1773,

SEQ ID NO: 17

left Primer: 1802, 1806, 1808, 1809, 1811, 1812, 1813, 1814,

right Primer: 1801, 1815, 1816, 1817, 1818, 1820, 1822,

Blocker: 1805, 1819, 1821, 1823, 1824, 1825, 1826, 1827, 1828, 1829,

Detection: 1803,

Anchor: 1804, 1807, 1810,

SEQ ID NO: 21

left Primer: 1831, 1835, 1837, 1838, 1839, 1840, 1841, 1842, 1843, 1844, 1845, 1846,
1847, 1848, 1849, 1850, 1851, 1852, 1853, 1854, 1855, 1856, 1857, 1858, 1859, 1860, 1861,
1862, 1863, 1864, 1865, 1866, 1868, 1869, 1870, 1872, 1873, 1874, 1875, 1876, 1877, 1878,
1879, 1880, 1881, 1882, 1883, 1884, 1887, 1889, 1890, 1891, 1895,

right Primer: 1830, 1885, 1886, 1888, 1892, 1893, 1894, 1897, 1898,

Blocker: 1834, 1896,

Detection: 1832,

Anchor: 1833, 1836, 1867, 1871,

SEQ ID NO: 35

left Primer: 1900, 1904, 1905, 1906,

right Primer: 1899, 1907, 1909, 1910, 1912, 1913, 1914, 1915, 1916, 1918, 1919,
1920,

Blocker: 1903, 1917, 1921,

Detection: 1901,

Anchor: 1902, 1908, 1911,

SEQ ID NO: 25

left Primer: 1923,

right Primer: 1922, 1927, 1928, 1929,

Blocker: 1926, 1930, 1931, 1932, 1933, 1934,

Detection: 1924,

Anchor: 1925,

SEQ ID NO: 26

left Primer: 1936, 1940, 1941, 1942, 1944, 1945, 1947, 1948, 1950, 1951, 1952,

right Primer: 1935, 1953, 1954, 1955, 1958, 1959, 1960, 1961, 1962, 1963, 1964, 1965, 1967, 1968, 1969, 1970, 1971, 1972,

Blocker: 1939, 1966, 1973, 1974, 1975,

Detection: 1937,

Anchor: 1938, 1943, 1946, 1949, 1956, 1957,

SEQ ID NO: 38

left Primer: 1977, 1981, 1982, 1983, 1984, 1985, 1987, 1988, 1989, 1990, 1991, 1993, 1994, 1995, 1996, 1997, 1999, 2000, 2002, 2004, 2005, 2006, 2007, 2008, 2009, 2011, 2012,

right Primer: 1976, 2013, 2014, 2015, 2016, 2017, 2019, 2021, 2024,

Blocker: 1980, 2018, 2022, 2023, 2026,

Detection: 1978,

Anchor: 1979, 1986, 1992, 1998, 2001, 2003, 2010, 2020, 2025,

SEQ ID NO: 27

left Primer: 2028, 2032, 2033, 2034, 2035, 2036, 2037, 2038, 2039, 2040, 2041, 2042, 2043, 2044, 2045, 2046, 2047, 2048, 2049, 2050, 2051, 2052, 2053, 2054, 2055, 2056, 2057, 2058, 2059, 2060, 2061, 2062, 2063, 2064, 2065, 2066, 2067, 2068, 2069, 2070, 2071, 2072, 2073, 2074, 2075, 2076, 2077,

right Primer: 2027, 2078, 2079, 2080, 2081, 2083, 2084, 2085, 2086, 2088, 2090, 2091, 2092, 2093, 2098, 2100, 2101, 2102, 2103, 2104, 2105, 2106, 2108, 2109, 2110,

Blocker: 2031, 2082, 2087, 2089, 2094, 2095, 2096, 2097, 2099, 2107,

Detection: 2029,

Anchor: 2030,

Taqman Real-time single probe detection assays:

SEQ ID NO: 41

left Primer: 2112, 2115, 2116, 2117,

right Primer: 2111, 2118, 2120, 2121, 2122, 2123, 2126, 2128, 2129, 2130, 2131, 2134, 2136, 2145,

Blocker: 2114, 2119, 2124, 2125, 2127, 2132, 2133, 2135, 2137, 2138, 2139, 2140, 2141, 2142, 2143, 2144, 2146, 2147, 2148, 2149, 2150, 2151, 2152, 2153, 2154, 2155, 2156, 2157, 2158, 2159, 2160,

Detection: 2113,

SEQ ID NO: 6

left Primer: 2162, 2165, 2166, 2167, 2168, 2169, 2170, 2171, 2172, 2173,

right Primer: 2161, 2174, 2175, 2176, 2177, 2178, 2179, 2181, 2182, 2184, 2185, 2186,

Blocker: 2164, 2180, 2183, 2187, 2188, 2189, 2190, 2191, 2192, 2193, 2194, 2195, 2196, 2197, 2198,

Detection: 2163,

SEQ ID NO: 8

left Primer: 2199, 2203,

right Primer: 2200,

Blocker: 2202, 2204, 2205, 2206,

Detection: 2201,

SEQ ID NO: 14

left Primer: 2208, 2211, 2212, 2213, 2214,

right Primer: 2207, 2215, 2216, 2217, 2220, 2224, 2225, 2226,

Blocker: 2210, 2218, 2219, 2221, 2222, 2223, 2227, 2228,

Detection: 2209,

SEQ ID NO: 9

left Primer: 2230, 2233, 2234, 2235, 2236, 2237, 2238, 2239, 2240, 2241, 2242, 2243, 2244,

right Primer: 2229, 2247, 2253,

Blocker: 2232, 2245, 2246, 2248, 2249, 2250, 2251, 2252, 2254, 2255, 2256,

Detection: 2231,

SEQ ID NO: 46

left Primer: 2257, 2264, 2265, 2266, 2267, 2269,

right Primer: 2258, 2261, 2262, 2263,

Blocker: 2260, 2268, 2270, 2271, 2272, 2273, 2274, 2275, 2276, 2277, 2278, 2279, 2280, 2281, 2282, 2283, 2284, 2285, 2286, 2287, 2288, 2289, 2290, 2291, 2292, 2293, 2294, 2295, 2296, 2297, 2298, 2299, 2300, 2301,

Detection: 2259,

SEQ ID NO: 13

left Primer: 2303, 2306, 2307, 2308, 2309, 2310, 2311, 2312, 2313,

right Primer: 2302, 2314, 2316, 2317, 2319, 2320, 2321, 2322, 2323,

Blocker: 2305, 2315, 2318,

Detection: 2304,

SEQ ID NO: 18

left Primer: 2325, 2328, 2329, 2330,

right Primer: 2324, 2331, 2332,

Blocker: 2327, 2333, 2334, 2335,

Detection: 2326,

SEQ ID NO: 20

left Primer: 2337, 2340, 2341, 2342, 2343, 2344, 2345, 2346, 2366, 2368, 2371, 2381, 2383, 2384, 2385, 2388, 2390, 2391, 2392, 2399, 2400, 2401,

right Primer: 2336, 2347, 2350, 2352, 2353, 2354, 2355, 2356, 2357, 2358, 2359, 2360, 2361, 2362, 2363, 2364, 2365,

Blocker: 2339, 2349, 2367, 2370, 2372, 2373, 2374, 2375, 2376, 2377, 2378, 2379, 2380, 2382, 2386, 2387, 2389, 2393, 2394, 2395, 2396, 2397, 2398, 2402, 2403, 2404,

Detection: 2338, 2348, 2351, 2369,

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SEQ ID NO: 48

left Primer: 2406, 2409, 2410, 2411, 2412, 2413, 2414, 2415, 2416, 2417, 2418, 2419, 2420, 2421, 2422, 2423, 2424, 2425, 2426, 2427, 2428, 2429, 2430, 2431, 2432, 2433, 2434, 2435, 2436, 2437, 2438, 2439, 2440, 2441, 2442, 2443, 2444, 2445, 2446, 2447, 2448, 2449, 2450, 2451, 2452, 2453, 2454, 2455, 2456, 2457, 2458, 2459, 2460, 2461, 2462, 2463,

right Primer: 2405,

Blocker: 2408, 2464, 2465, 2466, 2467, 2468,

Detection: 2407,

SEQ ID NO: 22

left Primer: 2469, 2483, 2485,

right Primer: 2470, 2473, 2474, 2475, 2476, 2477, 2478, 2479, 2480, 2481,

Blocker: 2472, 2482, 2484,

Detection: 2471,

SEQ ID NO: 19

left Primer: 2486, 2494, 2495, 2496, 2502, 2503, 2506, 2509, 2516, 2517, 2518, 2523, 2524, 2533, 2538, 2548, 2549, 2556,

right Primer: 2487, 2490, 2491, 2492, 2493, 2555, 2559, 2560, 2561, 2562, 2565, 2566, 2567, 2568, 2569, 2570, 2571, 2573, 2574, 2575, 2576, 2577, 2578, 2579,

Blocker: 2489, 2497, 2498, 2499, 2500, 2501, 2504, 2505, 2507, 2508, 2510, 2511, 2512, 2513, 2514, 2515, 2519, 2520, 2521, 2522, 2525, 2526, 2527, 2528, 2529, 2530, 2531, 2532, 2534, 2535, 2536, 2537, 2539, 2540, 2541, 2542, 2543, 2544, 2545, 2546, 2547, 2550, 2551, 2552, 2553, 2554, 2558, 2563, 2564, 2572, 2580, 2581,

Detection: 2488, 2557,

SEQ ID NO: 17

left Primer: 2583, 2586, 2587, 2588, 2589, 2590, 2591,

right Primer: 2582, 2592, 2593,

Blocker: 2585, 2594, 2595, 2596, 2597, 2598, 2599,

Detection: 2584,

SEQ ID NO: 21

left Primer: 2600, 2612, 2615, 2618, 2619, 2620, 2621, 2622, 2623, 2624, 2625, 2626, 2627, 2628, 2629, 2630, 2631, 2632, 2633, 2634, 2635, 2636, 2637, 2638, 2639, 2640, 2641, 2642, 2643, 2644, 2645, 2646, 2647,

right Primer: 2601, 2604, 2605, 2606, 2607, 2608, 2609, 2610, 2611, 2614, 2648, 2649, 2650, 2651, 2652, 2653, 2654,

Blocker: 2603, 2613, 2617,

Detection: 2602, 2616,

SEQ ID NO: 36

left Primer: 2655, 2659, 2676, 2677, 2678, 2680,

right Primer: 2656, 2660, 2663, 2665, 2666, 2667, 2668, 2669, 2670, 2672, 2673, 2674, 2675,

Blocker: 2658, 2662, 2679,

Detection: 2657, 2661, 2664, 2671,

SEQ ID NO: 26

left Primer: 2682, 2685, 2686, 2687, 2688, 2689,

right Primer: 2681, 2690, 2691, 2692, 2693, 2694, 2695, 2696, 2697, 2698, 2700, 2701, 2702,

Blocker: 2684, 2699, 2703, 2704, 2705, 2706,

Detection: 2683,

SEQ ID NO: 38

left Primer: 2708, 2711, 2712, 2713, 2714, 2715,

right Primer: 2707, 2716,

Blocker: 2710, 2717, 2718, 2719, 2720, 2721,

Detection: 2709,

SEQ ID NO: 27

left Primer: 2723, 2726, 2727, 2728, 2729, 2730, 2731, 2732, 2733, 2734, 2735, 2736, 2737, 2738, 2739, 2740, 2741, 2742, 2743, 2745,

right Primer: 2722, 2744, 2746, 2747,

Blocker: 2725, 2748, 2749, 2750,

Detection: 2724,

Additional embodiments of the invention provide a method for the analysis of the methylation status of genomic DNA according to the invention (SEQ ID NOS:1 to SEQ ID NO:58, and complements thereof) without the need for pretreatment.

In the *first step* of such additional embodiments, the genomic DNA sample is isolated from tissue or cellular sources. Preferably, such sources include cell lines, histological slides, body fluids, or tissue embedded in paraffin. In the *second step*, the genomic DNA is extracted. Extraction may be by means that are standard to one skilled in the art, including but not limited to the use of detergent lysates, sonification and vortexing with glass beads. Once the nucleic acids have been extracted, the genomic double-stranded DNA is used in the analysis.

In a preferred embodiment, the DNA may be cleaved prior to the treatment, and this may be by any means standard in the state of the art, in particular with methylation-sensitive restriction endonucleases.

In the *third step*, the DNA is then digested with one or more methylation sensitive restriction enzymes. The digestion is carried out such that hydrolysis of the DNA at the restriction site is informative of the methylation status of a specific CpG dinucleotide.

In the *fourth step*, which is optional but a preferred embodiment, the restriction fragments are amplified. This is preferably carried out using a polymerase chain reaction, and said amplificates may carry suitable detectable labels as discussed above, namely fluorophore labels, radionuclides and mass labels.

In the *fifth step* the amplificates are detected. The detection may be by any means standard in the art, for example, but not limited to, gel electrophoresis analysis, hybridization analysis, incorporation of detectable tags within the PCR products, DNA array analysis, MALDI or ESI analysis.

In the final step the of the method the presence, absence or subclass of colon cell proliferative disorder is deduced based upon the methylation state of at least one CpG dinucleotide sequence of SEQ ID NOS:1 to SEQ ID NO:58 , or an average, or a value

reflecting an average methylation state of a plurality of CpG dinucleotide sequences of SEQ ID NOS:1 to SEQ ID NO:58.

In a particularly preferred embodiment of the method the fourth step of the method comprises the use of at least one pair of MSP primers, and the use of hybridization probes for the detection of the subsequant amplificates by means of a real time assay.

Diagnostic and/or Prognostic Assays for colon cell proliferative disorders

The present invention enables diagnosis and/or prognosis of events which are disadvantageous to patients or individuals in which important genetic and/or epigenetic parameters within one or more of SEQ ID NOS:1 to SEQ ID NO:58 may be used as markers. Said parameters obtained by means of the present invention may be compared to another set of genetic and/or epigenetic parameters, the differences serving as the basis for a diagnosis and/or prognosis of events which are disadvantageous to patients or individuals.

Specifically, the present invention provides for diagnostic and/or prognostic cancer assays based on measurement of differential methylation of one or more CpG dinucleotide sequences of SEQ ID NOS:1 to SEQ ID NO:58, or of subregions thereof that comprise such a CpG dinucleotide sequence. Typically, such assays involve obtaining a tissue sample from a test tissue, performing an assay to measure the methylation status of at least one of one or more CpG dinucleotide sequences of SEQ ID NOS:1 to SEQ ID NO:58 derived from the tissue sample, relative to a control sample, or a known standard and making a diagnosis or prognosis based thereon.

In particular preferred embodiments, inventive oligomers are used to assess the CpG dinucleotide methylation status, such as those based on SEQ ID NOS:1 to SEQ ID NO:535, or arrays thereof, as well as in kits based thereon and useful for the diagnosis and/or prognosis of colon cell proliferative disorders.

Kits

Moreover, an additional aspect of the present invention is a kit comprising, for example: a bisulfite-containing reagent; a set of primer oligonucleotides containing at least

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two oligonucleotides whose sequences in each case correspond, are complementary, or hybridize under stringent or highly stringent conditions to a 16-base long segment of the sequences SEQ ID NOs:1 to SEQ ID NO:535; oligonucleotides and/or PNA-oligomers; as well as instructions for carrying out and evaluating the described method. In a further preferred embodiment, said kit may further comprise standard reagents for performing a CpG position-specific methylation analysis, wherein said analysis comprises one or more of the following techniques: MS-SNuPE, MSP, MethyLight ™, HeavyMethyl™, COBRA, and nucleic acid sequencing. However, a kit along the lines of the present invention can also contain only part of the aforementioned components.

While the present invention has been described with specificity in accordance with certain of its preferred embodiments, the following example serves only to illustrate the invention and is not intended to limit the invention within the principles and scope of the broadest interpretations and equivalent configurations thereof.

EXAMPLES

Samples were received either as frozen tissue or extracted genomic DNA. DNA samples were extracted using lysis buffer from Qiagen and the Roche magnetic separation kit for genomic DNA isolation. DNA samples were also extracted using Qiagen Genomic Tip-100 columns, as well as the MagnaPure device and Roche reagents. All samples were quantitated using spectrophotometric or fluorometric techniques and on agarose gels for a subset of samples.

Bisulfite treatment and mPCR

Total genomic DNA of all samples was bisulfite treated converting unmethylated cytosines to uracil. Methylated cytosines remained conserved. Bisulfite treatment was performed with minor modifications according to the protocol described in Olek et al. (1996). In order to avoid processing all samples with the same biological background together resulting in a potential process-bias in the data later on, the samples were randomly grouped into processing batches. For bisulfite treatment we created batches of 50 samples randomized for sex, diagnosis, and tissue. Per DNA sample two independent bisulfite reactions were

performed. After bisulfitation 10 ng of each DNA sample was used in subsequent mPCR reactions containing 6-8 primer pairs.

Each reaction contained the following:

0.4 mM each dNTPS

1 Unit Taq Polymerase

2.5 µl PCR buffer

3.5 mM MgCl2

80 nM Primerset (12-16 primers)

11.25 ng DNA (bisulfite treated)

Further details of the primers are shown in TABLE 1.

Forty cycles were carried out as follows: Denaturation at 95°C for 15 min, followed by annealing at 55°C for 45 sec., primer elongation at 65°C for 2 min. A final elongation at 65°C was carried out for 10 min.

1.1.2 Hybridization

All PCR products from each individual sample were then hybridised to glass slides carrying a pair of immobilised oligonucleotides for each CpG position under analysis. Each of these detection oligonucleotides was designed to hybridise to the bisulphite converted sequence around one CpG site which was either originally unmethylated (TG) or methylated (CG). See Table 2 for further details of all hybridisation oligonucleotides used (both informative and non-informative.) Hybridisation conditions were selected to allow the detection of the single nucleotide differences between the TG and CG variants.

5 µl volume of each multiplex PCR product was diluted in 10 x Ssarc buffer (10 x Ssarc:230 ml 20 x SSC, 180 ml sodium lauroyl sarcosinate solution 20%, dilute to 1000 ml with dH2O). The reaction mixture was then hybridised to the detection oligonucleotides as follows. Denaturation at 95°C, cooling down to 10 °C, hybridisation at 42°C overnight followed by washing with 10 x Ssarc and dH2O at 42°C.

Further details of the hybridisation oligonucleotides are shown in TABLE 2.

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Fluorescent signals from each hybridised oligonucleotide were detected using genepix scanner and software. Ratios for the two signals (from the CG oligonucleotide and the TG oligonucleotide used to analyse each CpG position) were calculated based on comparison of intensity of the fluorescent signals.

The samples were processed in batches of 80 samples randomized for sex, diagnosis, tissue, and bisulphite batch For each bisulfite treated DNA sample 2 hybridizations were performed. This means that for each sample a total number of 4 chips were processed.

Data analysis methods

Analysis of the chip data:

From raw hybridization intensities to methylation ratios;

The log methylation ratio (log(CG/TG)) at each CpG position is determined according to a standardized preprocessing pipeline that includes the following steps:

For each spot the median background pixel intensity is subtracted from the median foreground pixel intensity (this gives a good estimate of background corrected hybridization intensities):

For both CG and TG detection oligonucleotides of each CpG position the background corrected median of the 4 redundant spot intensities is taken;

For each chip and each CpG position the log(CG/TG) ratio is calculated;

For each sample the median of log(CG/TG) intensities over the redundant chip repetitions is taken.

This ratio has the property that the hybridization noise has approximately constant variance over the full range of possible methylation rates (Huber et al., 2002).

Principle Component Analysis

The principle component analysis (PCA) projects measurement vectors (*e.g.* chip data, methylation profiles on several CpGs etc.) onto a new coordinate system. The new coordinate axes are referred to as principal components. The first principal component spans the direction of the largest variance of the data. Subsequent components are ordered by

decreasing variance and are orthogonal to each other. Different CpG positions contribute with different weights to the extension of the data cloud along different components. PCA is an unsupervised technique, *i.e.*, it does not take into account the labels of the data points (for further details see e.g. Ripley (1996)).

PCA is typically used to project high dimensional data (in our case methylation-array data) onto lower dimensional subspaces in order to visualize or extract features with high variance from the data. In the present report we use 2 dimensional projections for statistical quality control of the data. We investigate the effect of different process parameters on the chip data and exclude that changing process parameters cause large alterations in the measurement values.

A robust version of PCA is used to detect single outlier chips and exclude them from further analysis (Model et al., 2002).

Hypothesis testing

The main task is to identify markers that show significant differences in the average degree of methylation between two classes. A significant difference is detected when the nullhypothesis that the average methylation of the two classes is identical can be rejected with $p<0.05$. Because we apply this test to a whole set of potential markers we have to correct the p-values for multiple testing. This was done by applying the False Discovery Rate (FDR) method (Dudoit et al., 2002).

For testing the null hypothesis that the methylation levels in the two classes are identical we used the likelihood ratio test for logistic regression models (Venables and Ripley, 2002). The logistic regression model for a single marker is a linear combination of methylation measurements from all CpG positions in the respective genomic region of interest (ROI). A significant p-value for a marker means that this ROI has some systematic correlation to the question of interest as given by the two classes. However, at least formally it makes no statement about the actual predictive power of the marker.

Class prediction by supervised learning

In order to give a reliable estimate of how well the CpG ensemble of a selected marker can differentiate between different tissue classes we can determine its prediction accuracy by classification. For that purpose we calculate a methylation profile based prediction function using a certain set of tissue samples with their class label. This step is called training and it exploits the prior knowledge represented by the data labels. The prediction accuracy of that function is then tested by cross-validation or on a set of independent samples. As a method of choice, we use the support vector machine (SVM) algorithm (Duda (2001), Christiannini (2000)) to learn the prediction function. If not stated otherwise, for this report the risk associated with false positive or false negative classifications are set to be equal relative to the respective class sizes. It follows that the learning algorithm obtains a class prediction function with the objective to optimize accuracy on an independent test sample set. Therefore sensitivity and specificity of the resulting classifier can be expected to be approximately equal.

Estimating the performance of the tissue class prediction: Cross Validation

With limited sample size the cross-validation method provides an effective and reliable estimate for the prediction accuracy of a discriminator function and therefore in addition to the significance of the markers we provide cross-validation accuracy, sensitivity and specificity estimates. For each classification task, the samples were partitioned into 5 groups of approximately equal size. Then the learning algorithm was trained on 4 of these 5 sample groups. The predictor obtained by this method was then tested on the remaining group of independent test samples. The number of correct positive and negative classifications was counted over 5 runs for the learning algorithm for all possible choices of the independent test group without using any knowledge obtained from the previous runs. This procedure was repeated on up to 10 random permutations of the sample set. Note that the above-described cross-validation procedure evaluates accuracy, sensitivity and specificity using practically all possible combinations of training and independent test sets. It therefore gives a better estimate of the prediction performance than simply splitting the samples into one training sample set and one independent test set.

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<u>Results</u>

Figures 1, 5, 9, 13, 16, 20, 24, 28 and 32 show ranked matrices of data obtained according to Examples 1 and 2 according to CpG methylation differences between the two classes of tissues, using an algorithim. The figures are shown in greyscale, wherein the most significant CpG positions are at the bottom of the matrix with significance decreasing towards the top. Black indicates total methylation at a given CpG position, white represents no methylation at the particular position, with degrees of methylation represented in grey, from light (low proportion of methylation) to dark (high proportion of methylation). Each row represents one specific CpG position within a gene and each column shows the methylation profile for the different CpGs for one sample. On the right side p values for the individual CpG positions are shown. The p values are the probabilities that the observed distribution occurred by chance in the data set.

Figures 2, 6, 10, 17, 21, 25, 29 and 33 show the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figures 3, 7, 11, 14, 18, 22, 26, 30 and 34 show ranked matrices of data obtained according to Examples 1 and 2 of the accuracy of the genewise linear support vector machine cross validations between the two classes of tissues, for the best performing markers. The figures are shown in greyscale, wherein the most significant CpG positions are at the bottom of the matrix with significance decreasing towards the top. Black indicates total methylation at a given CpG position, white represents no methylation at the particular position, with degrees of methylation represented in grey, from light (low proportion of methylation) to dark (high proportion of methylation). Each row represents one specific CpG position within a gene and each column shows the methylation profile for the different CpGs for one sample. On the right side accuracy values for each individual genomic region of interest are shown.

Figures 4, 8, 12, 15, 18, 23, 27, 31 and 35 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification. The accuracy of each genomic region is represented as black squares, the specificity as unfilled

diamonds, the sensitivity as unfilled squares. The accuracy as measured on the X-axis shows the fraction of correctly classified samples.

Colon Normal vs. Colorectal Cancer

In the first comparison 102 colorectal carcinoma samples were compared to 73 samples from normal colon, including colon polyps and colon inflammatory disorders.

Figure 1 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 2 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figure 3 shows the accuracy of the top 12 performing markers.

Figure 4 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:
SEQ ID NOS:1-12, 15-20, 22, 25-36, 38-49, 51-58, and complements thereof.

Other Tissues vs. Colorectal Cancer

In this classification 73 colorectal carcinoma samples were compared to an 'other tissue' class consisting of 140 samples from non-colorectal carcinomas, peripheral blood lymphocytes and other normal tissues of non-colorectal origin. These markers therefore enable the detection of colorectal carcinoma cells in', for example, body fluids such as serum.

Figure 5 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 6 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figure 7 shows the accuracy of the top 12 performing markers.

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Figure 8 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:1-23, 26-36, 38-43, 45-49, 51-58 and complements thereof.

Colon Normal and Other Tissue vs. Colon Cancer

In this classification 73 colorectal carcinoma samples were compared to 242 colon normal and 'other tissue' samples. The colon normal class consisted of healthy colon, colon polyps and inflammatory disorder colon tissue samples, the 'other tissues' consisted of samples from non-colorectal carcinomas, peripheral blood lymphocytes and other normal tissues of non-colorectal origin.

Figure 9 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 10 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figure 11 shows the accuracy of the top 12 performing markers.

Figure 12 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:1-3, 5-13, 15-23, 26-36, 38-43, 45-49, 51-58, and complements thereof.

Polyps vs. Colorectal Cancer

In this classification 73 colorectal carcinoma samples were compared to 51 colon polyp samples.

Figure 13 shows the multivariate test results of the top performing markers using the conservative Bonferroni corrected LogReg test.

Figure 14 shows the accuracy of the top 12 performing markers.

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Figure 15 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:11, 25, 27, 38, 40, 45, 53, and complements thereof.

Colon normal vs. Colorectal cell proliferative disorder

In this calssification 124 colon cell proliferative disorder samples (consisting of colon polyps and colorectal carcinoma) were compared to 51 'normal colon' samples consisting of both healthy colon samples and colon tissue of inflammatory disorders.

Figure 16 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 17 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figure 18 shows the accuracy of the top 12 performing markers.

Figure 19 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:1-58, and complements thereof.

Colon Normal vs. Colorectal Cancer

In this classification 73 colorectal carcinoma samples were compared to 51 'normal colon' samples consisting of both healthy colon samples and colon tissue of inflammatory disorders.

Figure 20 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 21 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

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Figure 22 shows the accuracy of the top 12 performing markers.

Figure 23 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:1-3, 5-23, 25-36, 38-49, 51-58, and complements thereof.

Colon Normal and Other Tissues vs. Colon Cell Proliferative Disorder

In this classification 124 colon cell proliferative disorder samples (consisting of colon polyps and colorectal carcinoma) were compared to a class consisting of 'colon normal' and 'other tissue' samples. The colon normal samples consisted of both healthy colon samples and colon tissue of inflammatory disorders, the 'other tissue' samples consisted of samples from non-colorectal carcinomas, peripheral blood lymphocytes and other normal tissues of non-colorectal origin.

Figure 24 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 25 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figure 26 shows the accuracy of the top 12 performing markers.

Figure 27 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:1-36, 38-43, 45-58, and complements thereof.

Other Tissue vs. Colon Tissue

The following comparison was carried out in order to identify markers capable of discerning elevated levels of free floating colon DNA, especially in bodily fluids as a marker of tumor progression. In this classification the 'colon tissue' class consisted of samples from colorectal carcinoma, colon polyps, colon tissue of inflammatory disorders and healthy colon

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tissue. The 'other tissue' class consisted of samples from non-colorectal carcinomas, peripheral blood lymphocytes and other normal tissues of non-colorectal origin.

Figure 28 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 29 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figure 30 shows the accuracy of the top 12 performing markers.

Figure 31 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:1-58, and complements thereof.

Normal Tissue vs. Cell Proliferative Disorder Tissue

In this classification the gene panel was assessed for its ability to accurately discriminate cell proliferative disorder samples from both colorectal carcinoma, colon polyps and non-colon origin cancers from 'normal tissues', namely healthy colon samples, colon tissue of inflammatory disorders, peripheral blood lymphocytes, other normal tissues of non-colorectal origin.

Figure 32 shows the multivariate test results of the top 12 performing markers using the conservative Bonferroni corrected LogReg test.

Figure 33 shows the uncorrected multivariate LogReg test results for each comparison. Each individual genomic region of interest is represented as a point, the dotted line represents the cut off point for the 25% false discovery rate.

Figure 34 shows the accuracy of the top 12 performing markers.

Figure 35 shows the accuracy of the genewise linear support vector machine cross validation for all genomic regions of each classification.

The following genomic markers were evaluated as being significant:

SEQ ID NOS:1-36, 38-43, 45-49, 51-58, and complements thereof.

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The following examples describe the analysis of the methylation status of the genomic sequences SEQ ID NO:35, SEQ ID NO:34 , SEQ ID NO:39, SEQ ID NO:29 in healthy and sick colon cell proliferative disorder samples. The initial link between said genes and colon cell proliferative disorders was initially carried by means of hybridisation analysis as described in EXAMPLE 1. The sequences were then selected from the larger set of genes analysed in said example, and the correlation between methylation status and colon cell proliferative disorder states was validated by analysis of samples using other methylation analysis techniques, namely the MSP- MethyLight ™ and HeavyMethyl ™ MethyLight ™ assays. Please note that the term 'MethyLight ™' is used to describe real time PCR analysis of bisulfite treated DNA using probes of both the Taqman ™ (single probe) and Lightcycler ™ (dual probe) technologies.

EXAMPLE 2

Analysis of methylation within colon cancer using an MSP- MethyLight assay (SEQ ID NO 35) DNA was extracted from 33 colon adenocarcinoma samples and 43 colon normal adjacent tissues using a Qiagen extraction kit. The DNA from each sample was treated using a bisulfite solution (hydrogen sulfite, disulfite) according to the agarose-bead method (Olek et al 1996). The treatment is such that all non methylated cytosines within the sample are converted to thymidine. Conversely, 5-methylated cytosines within the sample remain unmodified.

The methylation status was determined with a MSP-MethyLight assay designed for the CpG island of interest and a control fragment from the *beta* actin gene (Eads et al., 2001). The CpG island assay covers CpG sites in both the primers and the Taqman style probe, while the control gene does not. The control gene is used as a measure of total DNA concentration, and the CpG island assay (methylation assay) determines the methylation levels at that site.

Methods: The SEQ ID NO 35 gene CpG island assay was performed using the following primers and probes:

Forward Primer: CGGAGGGTACGGAGATTACG (SEQ ID NO:14387);

Reverse Primer: CGACGACGCGCGAAA (SEQ ID NO:14388); and

Probe: CGAAACCCTAAATATCCCGAATAACGCCG (SEQ ID NO: 14389).The corresponding control assay was performed using the following primers and probes:

Primer: TGGTGATGGAGGAGGTTTAGTAAGT (SEQ ID NO:14390);

Primer: AACCAATAAAACCTACTCCTCCCTTAA (SEQ ID NO:14391); and

Probe: ACCACCACCCAACACACAATAACAAACACA (SEQ ID NO:14392)

The reactions were run in triplicate on each DNA sample with the following assay conditions:*Reaction solution*: (900 nM primers; 300 nM probe; 3.5 mM Magnesium Chloride; 1 unit of taq polymerase; 200 μM dNTPs; 7μl of DNA, in a final reaction volume of 20 μl);*Cycling conditions*: (95°C for 10 minutes; then 50 cycles of: 95°C for 15 seconds; 60°C for 1 minute).

The data was analysed using a PMR calculation previously described in the literature (Eads et al 2001).*Results*. The mean PMR for normal samples was 0.15, with a standard deviation of 0.18. The mean PMR for tumour samples was 17.98, with a standard deviation of 18.18. The overall difference in methylation levels between tumour and normal samples is significant in a t-test (p=0.00000312). The results are shown in Figure 36. A Receiver Operating Characteristic curve (ROC curve) of the assay was also determined. A ROC is a plot of the true positive rate against the false positive rate for the different possible cutpoints of a diagnostic test. It shows the tradeoff between sensitivity and specificity depending on the selected cutpoint (any increase in sensitivity will be accompanied by a decrease in specificity). The area under an ROC curve (AUC) is a measure for the accuracy of a diagnostic test (the larger the area the better, optimum is 1, a random test would have a ROC curve lying on the diagonal with an area of 0.5; for reference: J.P. Egan. Signal Detection Theory and ROC Analysis, Academic Press, New York, 1975). The AUC for the MSP-MethylLight™-Assay is: 0.94 (Figure 37).

EXAMPLE 3

Methylation of SEQ ID NO 35 within colon cancer was analysed using a HeavyMethyl MethyLight™ assay. The same DNA samples were also used to analyse

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methylation of the CpG island with a HeavyMethyl MethyLight™ (or HM MethyLight™) assay, also referred to as the HeavyMethyl™ assay. The methylation status was determined with a HM MethyLight™ assay designed for the CpG island of interest and the same control gene assay described above. The CpG island assay covers CpG sites in both the blockers and the Taqman™ style probe, while the control gene does not.

Methods. The CpG island assay (methylation assay) was performed using the following primers and probes:

Forward Primer: GGTGATTGTTTATTGTTATGGTTTG (SEQ ID NO:14393)

Reverse Primer: CCCCTCAACCTAAAAACTACAAC (SEQ ID NO:14394)

Forward Blocker: GTTATGGTTTGTGATTTTGTGTGGG (SEQ ID NO:14395)

Reverse Blocker: AAACTACAACCACTCAAATCAACCCA (SEQ ID NO:14396)

Probe: AAAATTACGACGACGCCACCCGAAA (SEQ ID NO:14397)

The reactions were each run in triplicate on each DNA sample with the following assay conditions:

Reaction solution: (400 nM primers; 400 nM probe; 10μM both blockers; 3.5 mM magnesium chloride; 1x ABI Taqman buffer; 1 unit of ABI TaqGold polymerase; 200μM dNTPs; and 7μl of DNA, in a final reaction volume of 20 μl);

Cycling conditions: (95°C for 10 minutes); (95°C for 15 seconds, 64°C for 1 minute (2 cycles)); (95°C for 15 seconds, 62°C for 1 minute (2 cycles); (95°C for 15 seconds, 60°C for 1 minute (2 cycles)); and (95°C for 15 seconds, 58°C for 1 minute, 60°C for 40 seconds (41 cycles)).

Results. The mean PMR for normal samples was 1.12 with a standard deviation of 1.45. The mean PMR for tumour samples was 38.23 with a standard deviation of 33.22. The overall difference in methylation levels between tumour and normal samples is significant in a t-test (p=0.000000326). The results are shown in Figure 36.

A ROC curve of the assay was also determined. The AUC for the MSP-Methyl-Light-Assay is 0.91 (Figure 38)

The assay was tested on an additional set of colon samples (25 adenocarcinoma, 33 normals, and 13 adenomas). The results showed a significant difference again (Figure 39). The ROC are shown in Figure 40-42.

The MSP and HeavyMethyl variants of the MethyLight assay were determined to be equivalent for the analysis of methylation in SEQ ID NO:35. Figure 48 shows the regression plot of the percentage methylation detected in each sample using the two methods.

EXAMPLE 4

The SEQ ID NO:35 -HeavyMethyl-MethyLight-assay was also tested against a panel of other tissues (Figure 43). Besides the colon cancer samples only one of the two breast cancer tissues were methylated. However, on a panel of 21 additional breast tumours (different stages), only one was methylated (Figure 44). So the marker is specific for colon tumour samples. All primers, probes, blockers and reaction conditions were identical to those used in the analysis of the colon cancer samples (Example 3).

EXAMPLE 5

Twelve of the colon tissues analysed by real-time PCR also had paired serum taken before surgery. We extracted DNA from 1 ml of that serum using a Qiagen UltraSens□ DNA extraction kit, bisulfite treated the DNA sample, and ran the SEQ ID NO:35 -HeavyMethyl-MethyLight™-assay on those samples. The control gene did not amplify for three of the cancer serum samples and three of the normal serum samples, so we can conclude that the sample preparation did not work in these cases. In the other cases, there was evidence of higher methylation in the cancer samples than the normal samples (Figure 45).

EXAMPLE 6

Analysis of methylation within colon cancer using a SEQ ID NO:34 -MSP-MethyLight™ Assay. The colon cancer samples described in Example 2 were also analysed using a SEQ ID NO:34 -MSP- MethyLight™ Assay with a Taqman® style probe. The sample

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preparation was carried out as described above (Example 1). The assay was performed using the following primers and probes:

Forward Primer: TGGGATTAAGATTTTCGGTTAGTTTC (SEQ ID NO:14398)

Reverse Primer: CACTACAACGCTACGCGACTAAA (SEQ ID NO:14399)

Probe: TCGACGTTACCCAAACGAATCACATAAAAAAC (SEQ ID NO:14400)

The corresponding control assay was performed as described above (EXAMPLE 2)

The reactions were run in triplicate on each DNA sample with the following assay conditions:

Reaction solution: (900 nM primers; 300 nM probe; 3.5 mM magnesium chloride; 1 units of taq polymerase; 200 μM dNTPs, 5μM blocker; and 7 μl of DNA, in a final reaction volume of 20 μl);

Cycling conditions: 95°C for 10 minutes; (95°C for 15 seconds, 60°C for 1 minute) 50 cycles

The data was analysed using a PMR calculation previously described in the literature (Eads et al 2001).

Results. The results are shown in Figure 36. The mean PMR for normal samples was 3.93, with a standard deviation of 3.57. The mean PMR for tumour samples was 23.06, with a standard deviation of 20.23 The overall difference in methylation levels between tumour and normal samples is significant in a t-test (p=0.000003063). The ROC curve of the assay is shown in Figure 46. The AUC is 0.84.

This was further confirmed using a SEQ ID NO:34 -HeavyMethyl MethyLight™ assay, using dual Lightcycler™ probes.

Methods. The CpG island assay (methylation assay) was performed using the following primers and probes:

Forward Primer: TGGATAGGAGTTGGGATTAAGATTTT (SEQ ID NO:14401)

Reverse Primer: CTTATTACAATTTAAAAAAAAAATTCACTACAA (SEQ ID NO:14402);

Blocker: AAATTCACTACAACACTACACAACTAAATTCAACATTAC (SEQ ID NO:14403);

Probe: TTTTCGTATTTTTTTCGGGTTATTACGTTTT-Fluor (SEQ ID NO:14404);

Probe: LC640-ATGTGATTCGTTTGGGTAACGTCGA-Phos (SEQ ID NO:14405.

The reactions were each run in triplicate on each DNA sample with the following assay conditions:

Reaction conditions: 500 nM primers; 10 μM blocker; 250 nM probes; 4 mM Magnesium Chloride

Cycling profile: 95°C denaturation for 10 minutes; 50 cycles: 95°C 10 seconds, 57°C 30 seconds, 72°C 20 seconds

EXAMPLE 7

Analysis of methylation within colon cancer using a SEQ ID NO:29 -MSP- MethyLight™ Assay. The colon cancer samples described in Example 2 were also analysed using a SEQ ID NO:29 -MSP- MethyLight™ Assay with a Taqman® style probe. The sample preparation was carried out as described above (Example 2). The assay was performed using the following primers and probes:

Forward Primer: TTTTTTTTTCGGACGTCGTTG (SEQ ID NO:14406)

Reverse Primer: CCTCTACATACGCCGCGAAT (SEQ ID NO:14407)

Probe: AATTACCGAAAACATCGACCGA (SEQ ID NO:14408)

The reactions were run in triplicate on each DNA sample with the following assay conditions:

Reaction solution: (900 nM primers; 300 nM probe; 3.5 mM magnesium chloride; 1 units of taq polymerase; 200 μM dNTPs, 5 μM blocker; and 7 μl of DNA, in a final reaction volume of 20 μl);

Cycling conditions: 95°C for 10 minutes; (95°C for 15 seconds, 60°C for 1 minute) 50 cycles

The corresponding control assay was performed as described above (EXAMPLE 2).

The data was analysed using a PMR calculation previously described in the literature (Eads et al 2001).

Results. The results are shown in Figure 36. The mean PMR for normal samples was 3.04, with a standard deviation of 4.21. The mean PMR for tumour samples was 21.38, with a

standard deviation of 24.08 The overall difference in methylation levels between tumour and normal samples is significant in a t-test (p=0.0000101973). The ROC curve of the assay is shown in Figure 47. The AUC is 0.80.

This was further confirmed using a SEQ ID NO:29 -HeavyMethyl MethyLight™ assay (using dual labeled Lightcycler™ probes.

Methods. The CpG island assay (methylation assay) was performed using the following primers and probes:

Forward Primer: GTAGGGTTATTGTTTGGGTTAATAAAT (SEQ ID NO:14409)

Reverse Primer: TAAAAAAAAAAAAAAAAAACTCCTCTACATAC (SEQ ID NO:14410)

Blocker: AACTCCTCTACATACACCACAAATAAATT (SEQ ID NO:14411)

Probe: CGAAAACATCGACCGAACAACG-Fluor (SEQ ID NO:14412)

Probe: LC640-GTCCGAAAAAAAAAAAACGAACTCC-Phos (SEQ ID NO:14413)

The reactions were each run in triplicate on each DNA sample with the following assay conditions:

Reaction conditions: Forward primer: 600 nM; Reverse primer: 30 nM; Blocker: 10 μM; Probes: 500 nM; Taq polymerase: 0.1 U/μl; dNTPs: 0.2mM each; Magnesium Chloride: 4 mM; BSA: 0.25 mg/ml; Roche buffer with no MgCl₂: 1x

Cycling conditions: 95°C denaturation for 10 minutes; 50 cycles: 95°C for 10 seconds, 57°C for 25 seconds, 72°C for 10 seconds

EXAMPLE 8

Analysis of methylation within colon cancer using a SEQ ID NO:29 -MSP-MethyLight™ Assay. An additional assay for SEQ ID NO:29 was tested on colon samples. The assay was tested on two sets of tissues, each with 12 colon adenocarcinomas and 12 normal adjacent tissue samples.

The sample preparation was carried out as described above (Example 2). The assay was performed using the following primers and probes:

Forward Primer: GGACGTTTTTTATCGAAGGCG (SEQ ID NO:14414)

Reverse Primer: GCCACCCAACCGCGA (SEQ ID NO:14415)

Probe: ACCCGAAATCACGCGCGAAAAA (SEQ ID NO:14416)

The reactions were run in triplicate on each DNA sample with the following assay conditions:

Reaction solution: (900 nM primers; 300 nM probe; 3.5 mM magnesium chloride; 1 units of taq polymerase; 200 µM dNTPs, 5 µM blocker; and 7 µl of DNA, in a final reaction volume of 20 µl);

Cycling conditions: 95°C for 10 minutes; (95°C for 15 seconds, 60°C for 1 minute) 50 cycles. The corresponding control assay was performed as described above (Example 2)

The data was analysed using a PMR calculation previously described in the literature (Eads et al 2001). In both cases, SEQ ID NO:29 was significantly more methylated in the cancer samples. The ROC curves of the assays are shown in Figures 49 and 50. The AUC are 0.93 and 1.

EXAMPLE 9

Analysis of methylation within colon cancer using a SEQ ID NO:39 -MSP-MethyLight™ Assay

The colon cancer samples described in Example 2 were also analysed using a SEQ ID NO:39 -MSP- MethyLight™ Assay. The sample preparation was carried out as described above (EXAMPLE 2). The assay was performed using the following primers and probes:

Forward Primer: GACGGATTTTTTTTTAACGTTTTTC (SEQ ID NO:14417)

Reverse Primer: AAATAAAATACCACCTCCGCGA (SEQ ID NO:14418)

Probe: GCTCCTCGCGAAATACTCACCCCG (SEQ ID NO:14419)

The reactions were run in triplicate on each DNA sample with the following assay conditions:

Reaction solution: (900 nM primers; 300 nM probe; 3.5 mM magnesium chloride; 1 units of taq polymerase; 200 µM dNTPs, 5 µM blocker; and 7 µl of DNA, in a final reaction volume of 20 µl);

Cycling conditions: 95°C for 10 minutes; (95°C for 15 seconds, 60°C for 1 minute) 50 cycles.

The corresponding control assay was performed as described above (EXAMPLE 2).

The data was analysed using a PMR calculation previously described in the literature (Eads et al 2001).

Results. The results are shown in Figure 36. The mean PMR for normal samples was 2.25, with a standard deviation of 2.42. The mean PMR for tumour samples was 25.67, with a standard deviation of 17.57 The overall difference in methylation levels between tumour and normal samples is significant in a t-test (p=0.0000000118). The ROC curve of the assay is shown in Figure 52. The AUC is 0.94

This was further confirmed using a SEQ ID NO:39 -HeavyMethyl MethyLight™ assay, using dual Lightcycler probes using Lightcycler style dual probe technology.

Methods. The CpG island assay (methylation assay) was performed using the following primers and probes:

Forward Primer: GTTAGTTAGTTAATTTTTTAAATAGATTAGTAG (SEQ ID NO:14420)

Reverse Primer: CAAAAAAACAAATAAAATACCACCTCC (SEQ ID NO:14421)

Blocker: CCTCCACAAAACTCACTCCTCACAAAATAC (SEQ ID NO:14422)

Probe: red640 TTTCGTTTTGTATGGTAGATACGGGGTGA- phosphate (SEQ ID NO:14423)

Probe: ATTAATGGTTTTATAAGACGGATTTTTTTTTAACGT- fluorescine (SEQ ID NO:14424)

The reactions were each run in triplicate on each DNA sample with the following assay conditions:

Reaction conditions: Forward primer: 600 nM; Reverse primer: 300 nM; Blocker: 10 µM; Probes: 500 nM; Taq polymerase: 0.1 U/µl; dNTPs: 0.2 mM each; Magnesium Chloride: 4 mM; BSA: 0.25 mg/ml; Roche buffer with no MgCl₂: 1x

Cycling conditions: 95°C denaturation for 10 minutes; 50 cycles: 95°C for 10 seconds, 57°C for 25 seconds, 72°C for 10 seconds.

TABLES

TABLE 1.

Gene:	Primer:	Amplificate Length:
(SEQ ID NO: 16)	TTGGAGTTAAAGTATTTGGTAAGA (SEQ ID NO: 536) AAAACCACCTTCAAACCC (SEQ ID NO: 537)	442
(SEQ ID NO: 4)	ATCCTCCACACTCTTCCTCTAT (SEQ ID NO: 538) GAAATTAGGTTTGGTTTTGTTT (SEQ ID NO: 539)	140
(SEQ ID NO: 4)	GAGATTTTGGGAGGGGTAG (SEQ ID NO: 540) AACTCTATCCTTTTCCCTCTTC (SEQ ID NO: 541)	486
(SEQ ID NO: 56)	TGTTGGTTGTTGTTGTTGTT (SEQ ID NO: 542) CTTTCTACCCATCCCAAAA (SEQ ID NO: 543)	319
(SEQ ID NO: 27)	TAAGTGATAAAGGAAGGAAGGA (SEQ ID NO: 544) CCTTCAAACCCCAAACAA (SEQ ID NO: 545)	243
(SEQ ID NO: 31)	TTGTTGTTTTTAGGGGTTTTGG (SEQ ID NO: 546) TCCTTCCCATTCTCCAAATATC (SEQ ID NO: 547)	947
(SEQ ID NO: 32)	TCAACTACCATCAACTTCCTTA (SEQ ID NO: 548) AATTTATTTTTAGTGTTGTAGTGGG (SEQ ID NO: 549)	491
(SEQ ID NO: 33)	GAAAGGAGAGGTTAAAGGTTG (SEQ ID NO: 550) AACTCACTTAACTCCAATCCC (SEQ ID NO: 551)	696
(SEQ ID NO: 34)	GGATAGGAGTTGGGATTAAGAT (SEQ ID NO: 552) AAATCTTTTTCAACACCAAAAT (SEQ ID NO: 553)	414
	TCCAATAAACACAAACCTAAATC	471

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Gene:	Primer:	Amplificate Length:
(SEQ ID NO: 24)	(SEQ ID NO: 554) ATATGGGATTGATGGAAGATAG (SEQ ID NO: 555)	
(SEQ ID NO: 35)	GGAAGAGGTGATTAAATGGAT (SEQ ID NO: 556) CCCAAAAATCAAACAACAA (SEQ ID NO: 557)	226
(SEQ ID NO: 57)	ATTTGGGAAAGAGGGAAAG (SEQ ID NO: 558) TAAAAACTCTAAACCCCATCC (SEQ ID NO: 559)	300
(SEQ ID NO: 25)	CCCTACCCACCAATATACC (SEQ ID NO: 560) AGATTTGGGGAAGAAGTTGTA (SEQ ID NO: 561)	278
(SEQ ID NO: 36)	TAAAAGAAGGATTTTTGATTGG (SEQ ID NO: 562) CATCTTATTTACCTCCCTCCC (SEQ ID NO: 563)	528
(SEQ ID NO: 28)	TTTTAGATTGAGGTTTTAGGGT (SEQ ID NO: 564) ATCCATTCTACCTCCTTTTTCT (SEQ ID NO: 565)	497
(SEQ ID NO: 37)	GTAATTTGAAGAAAGTTGAGGG (SEQ ID NO: 566) CCAACAACTAAACAAAACCTCT (SEQ ID NO: 567)	296
(SEQ ID NO: 26)	AGTAAATAGTGGGTGAGTTATGAA (SEQ ID NO: 568) GAAAAACCTCTAAAAACTACTCTCC (SEQ ID NO: 569)	607
(SEQ ID NO: 38)	GTTAGTATGTTTGGGGGTAAAT (SEQ ID NO: 570) ATAAATAACACCTTCCACCCTA (SEQ ID NO: 571)	435
(SEQ ID NO: 39)	TTTGTATTAGGTTGGAAGTGGT (SEQ ID NO: 572) CCCAAATAAATCAACAACAACA (SEQ ID NO: 573)	286
(SEQ ID NO: 29)	TTGTTTGGGTTAATAAATGGA (SEQ ID NO: 574) CTTCTCTCTTCTCCCCTCTC (SEQ ID NO: 575)	295
(SEQ ID NO: 40)	AATATAGGGAGGTTTAGGGTTT (SEQ ID NO: 576) TAACCATACATTTCTCATCCAA (SEQ ID NO: 577)	424
(SEQ ID NO: 41)	TTTTGGGGAATATAGGGTTT (SEQ ID NO: 578)	425

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Gene:	Primer:	Amplificate Length:
	TTCTCACATTTCTAACCACTTCT (SEQ ID NO: 579)	
(SEQ ID NO: 5)	CTCCTCCTTCCAACAAAAA (SEQ ID NO: 580)	487
(SEQ ID NO: 5)	GTTTAGAGGTTTTGGGATGATT (SEQ ID NO: 581) TGAATAGGGTGATATTTTAGTTAGG (SEQ ID NO: 582) ATAAATCATCCCAAAACCTCTA (SEQ ID NO: 583)	497
(SEQ ID NO: 6)	ATTTGGTTATTGGTTGAAGGTA (SEQ ID NO: 584) AATTTTTAATTTCTCAACACCTCT (SEQ ID NO: 585)	361
(SEQ ID NO: 6)	GAAGAGGTGTTGAGAAATTAAAA (SEQ ID NO: 586) CCCACCCTAACTTACCATAAA (SEQ ID NO: 587)	462
(SEQ ID NO: 8)	CAATTCCCCTTATTTCTCTAAA (SEQ ID NO: 588) AATTAGTTATGGTGTTGTGGGA (SEQ ID NO: 589)	339
(SEQ ID NO: 8)	TTCTATTAAAACCCAACTCCTC (SEQ ID NO: 590) ATAAGGGGAATTGTTGTAGGTT (SEQ ID NO: 591)	395
(SEQ ID NO: 42)	TACCATTCTTTCCTAAACATCC (SEQ ID NO: 592) GGGTTGGTGGAGAGGTAG (SEQ ID NO: 593)	148
(SEQ ID NO: 14)	GAAGATGAGAGGAGGTTGAGA (SEQ ID NO: 594) CCACACCACCTACTACAAAAT (SEQ ID NO: 595)	500
(SEQ ID NO: 15)	AACAAACCTCCTCCAAATTC (SEQ ID NO: 596) TGTTGGTAGGTATTGGTGATT (SEQ ID NO: 597)	365
(SEQ ID NO: 15)	TCCCCACTTAAAATAAACAAAT (SEQ ID NO: 598) GTGAATTTGGAGGAGGTTT (SEQ ID NO: 599)	375
(SEQ ID NO: 7)	GGGGTTGATATTGTTTTTAGAG (SEQ ID NO: 600) CCCCTCCTTCCTTAAATCT (SEQ ID NO: 601)	328
(SEQ ID NO: 44)	TTTTAGAAGGGGTTGGTTTAG (SEQ ID NO: 602) ACTACCTAACTCTCCCCACAA	343

Gene:	Primer:	Amplificate Length:
	(SEQ ID NO: 603)	
(SEQ ID NO: 44)	TTGTGGGGAGAGTTAGGTAGT (SEQ ID NO: 604) TAACCCAAATATCATAAAACCC (SEQ ID NO: 605)	411
(SEQ ID NO: 1)	AGATGGATATTTTGTTGGTGTT (SEQ ID NO: 606) TACACAATTATACCTTTCAAACAAT (SEQ ID NO: 607)	250
(SEQ ID NO: 1)	CCATACAAATATCCTAAATAAAACC (SEQ ID NO: 608) TTGTTGGAAGAATTGATAGTGTT (SEQ ID NO: 609)	482
(SEQ ID NO: 1)	AGGGAGTTAAGTAAGGGGTTAG (SEQ ID NO: 610) AACACCAACAAAATATCCATCT (SEQ ID NO: 611)	442
(SEQ ID NO: 2)	TGGAATTTTAGGGTTAGTAGGG (SEQ ID NO: 612) CAAATAAACCAAACCACTATCA (SEQ ID NO: 613)	499
(SEQ ID NO: 2)	TTGGGATTTTTAGGTGGTTT (SEQ ID NO: 614) TTCACTTTCCCTACTAACCCTA (SEQ ID NO: 615)	492
(SEQ ID NO: 45)	TGGGTTATTTGGTGAGTATTGT (SEQ ID NO: 616) CTTACCCCCACCCAACTA (SEQ ID NO: 617)	461
(SEQ ID NO: 46)	TTCACATTACATTAACCCATTTA (SEQ ID NO: 618) TTGGAGTTGTTAGGAGAAAAGT (SEQ ID NO: 619)	452
(SEQ ID NO: 10)	CCTTCCTTAAAAACCTCAAAAC (SEQ ID NO: 620) GTAAAGAATGGTAGAGGATAGGAT (SEQ ID NO: 621)	436
(SEQ ID NO: 10)	CTTACTACCCAACCTCTTTCAC (SEQ ID NO: 622) TGGAAGGATAGAGAATTTTGTT (SEQ ID NO: 623)	444
(SEQ ID NO: 11)	ATCCCATCTCTCAACTCCTACT (SEQ ID NO: 624) TGATTTATTTTGATGTGTGGTT (SEQ ID NO: 625)	452
(SEQ ID NO: 11)	TATTTAAGGATTTTGGAAGGAG (SEQ ID NO: 626) TCATCTCATTTTATCTCTACAACC (SEQ ID NO: 627)	349

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Gene:	Primer:	Amplificate Length:
(SEQ ID NO: 13)	AACAAATTCCCAACACACC (SEQ ID NO: 628) TTTTGGAAGATGGTTTATTTTT (SEQ ID NO: 629)	476
(SEQ ID NO: 13)	TTTTTAATATGGAGGTAAGGGA (SEQ ID NO: 630) AAATTCCCAACACACCAAC (SEQ ID NO: 631)	279
(SEQ ID NO: 3)	CTTCTCCAAAATCAACCAACT (SEQ ID NO: 632) TTTGTGTTATTAGTAGGTGAGAGG (SEQ ID NO: 633)	254
(SEQ ID NO: 3)	TTAGAAGTTGGAGGGTGAAAT (SEQ ID NO: 634) CTTCCTACCTTAAACCCTTACC (SEQ ID NO: 635)	450
(SEQ ID NO: 18)	TCTAACTCCTCACAAATTCCTAA (SEQ ID NO: 636) GTAGTGTAATAGGGAAAGGGG (SEQ ID NO: 637)	498
(SEQ ID NO: 48)	TAAAATTCCCTCTTACCCTAAA (SEQ ID NO: 638) TAGTAAGGATTGTAGAAGGGGG (SEQ ID NO: 639)	396
(SEQ ID NO: 48)	TAGTAAGGATTGTAGAAGGGGG (SEQ ID NO: 640) CCTCAAACCCTAAAAATAACC (SEQ ID NO: 641)	
(SEQ ID NO: 58)	GGAGAGGAGTGTTTGTAGAAGA (SEQ ID NO: 642) CAATCTCCCCTAAATCCTAAT (SEQ ID NO: 643)	369
(SEQ ID NO: 22)	TAGTAGTTTGAAGAAGGGGAAG (SEQ ID NO: 644) AAACATTCCTAAAATCACAAAAA (SEQ ID NO: 645)	373
(SEQ ID NO: 19)	TTTATTTGGGTATGATTAGGTTTT (SEQ ID NO: 646) ACTAAAAACACCACCCCCT (SEQ ID NO: 647)	426
(SEQ ID NO: 19)	ACAAACCAAAATCTTACTTCCTA (SEQ ID NO: 648) GAATGGAGGGGAAATGTTA (SEQ ID NO: 649)	458
(SEQ ID NO: 17)	AAAACTCCTCCCCTCTATAAAT (SEQ ID NO: 650) TTGGAGAGATGTGTTGGTTAG (SEQ ID NO: 651)	492
	AATCCTAACCAACACATCTCTC	482

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Gene:	Primer:	Amplificate Length:
(SEQ ID NO: 17)	(SEQ ID NO: 652) AGGGGATTTTAAGGTGATTAGT (SEQ ID NO: 653) CTCCCCATCCATCTTATTTTA	
(SEQ ID NO: 23)	(SEQ ID NO: 654) ATTGTTTGGGTGATAGTGAAGT (SEQ ID NO: 655)	489
(SEQ ID NO: 21)	TAAGTTTTTGGAGGAAGAGTTT (SEQ ID NO: 656) AAAATACTCCCTATAATTCCCC (SEQ ID NO: 657)	370
(SEQ ID NO: 21)	TTTCTCTAACCAAACACCTAAAA (SEQ ID NO: 658) AGAAATTAGTAGAGGAGGGAGG (SEQ ID NO: 659)	398
(SEQ ID NO: 43)	ATCTAATCCCTCTCCTAACTCC (SEQ ID NO: 660) TTTGTTTTGGAATTTAGGTTTT (SEQ ID NO: 661)	441
(SEQ ID NO: 9)	TCCACAAAACTCTCCTACTAAAA (SEQ ID NO: 662) GGAAGGTTGGGTAGATATAGG (SEQ ID NO: 663)	186
(SEQ ID NO: 12)	TTGGTAGAGTTGAAAGGAGATAG (SEQ ID NO: 664) AAAAACATTCCCTAAAAATTCC (SEQ ID NO: 665)	402
(SEQ ID NO: 47)	ATAGAATGGTTAGGGGGTATTT (SEQ ID NO: 666) TACAAATATCAACCTCTCTCCC (SEQ ID NO: 667)	484
(SEQ ID NO: 20)	GGTGGGGTATAATAGTAGGGAT (SEQ ID NO: 668) CTTCCCCTCTTTCATTTTATTT (SEQ ID NO: 669)	448
(SEQ ID NO: 50)	GAGGAATTGGTATTGAAAGAAA (SEQ ID NO: 670) CTAATCCACCCTCCATAAAAC (SEQ ID NO: 671)	426
(SEQ ID NO: 50)	CTCCAATTCTCCTCCCTATATC (SEQ ID NO: 672) TAATTTTTGAGGTTGGGAAA (SEQ ID NO: 673)	425

TABLE 2.

No:	Gene	Oligo:

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No:	Gene	Oligo:
1	(SEQ ID NO: 41)	TGGACGTAGGAAAGCGA (SEQ ID NO: 681)
2	(SEQ ID NO: 41)	GATGTAGGAAAGTGAGA (SEQ ID NO: 682)
3	(SEQ ID NO: 41)	ATTTACGGGAGTTTTATCGT (SEQ ID NO: 683)
4	(SEQ ID NO: 41)	ATTTATGGGAGTTTTATTGT (SEQ ID NO: 684)
5	(SEQ ID NO: 41)	ATTAGTTCGGGTCGCGT (SEQ ID NO: 685)
6	(SEQ ID NO: 41)	ATTAGTTTGGGTTGTGT (SEQ ID NO: 686)
7	(SEQ ID NO: 41)	TATACGAAAGGGAGGCGG (SEQ ID NO: 687)
8	(SEQ ID NO: 41)	TATATGAAAGGGAGGTGG (SEQ ID NO: 688)
9	(SEQ ID NO: 41)	GGCGTGTCGTTAGTTTTA (SEQ ID NO: 689)
10	(SEQ ID NO: 41)	GGTGTGTTGTTAGTTTTATA (SEQ ID NO: 690)
11	(SEQ ID NO: 41)	TTCGATTGACGTTAGCGA (SEQ ID NO: 691)
12	(SEQ ID NO: 41)	TTTGATTGATGTTAGTGA (SEQ ID NO: 692)
13	(SEQ ID NO: 41)	TTTCGAGTTTGACGGT (SEQ ID NO: 693)
14	(SEQ ID NO: 41)	TTTTGAGTTTGATGGTT (SEQ ID NO: 694)
15	(SEQ ID NO: 41)	TTCGGAGGGCGTATTT (SEQ ID NO: 695)
16	(SEQ ID NO: 41)	TTTGGAGGGTGTATTT (SEQ ID NO: 696)
17	(SEQ ID NO: 5)	GACGTCGGTACGTAGT (SEQ ID NO: 697)
18	(SEQ ID NO: 5)	GATGTTGGTATGTAGTAG (SEQ ID NO: 698)
19	(SEQ ID NO: 5)	TTCGGGGGAATTCGAGT (SEQ ID NO: 699)
20	(SEQ ID NO: 5)	TTTGGGGGAATTTGAGT (SEQ ID NO: 700)
21	(SEQ ID NO: 5)	TATTGCGAGGATTCGG (SEQ ID NO: 701)
22	(SEQ ID NO: 5)	ATTGTGAGGATTTGGT (SEQ ID NO: 702)
23	(SEQ ID NO: 5)	GTGCGTTCGTAGCGTA (SEQ ID NO: 703)
24	(SEQ ID NO: 5)	TGTGTTTGTAGTGTAGG (SEQ ID NO: 704)
25	(SEQ ID NO: 5)	GGACGTCGTTTGTTAG (SEQ ID NO: 705)

No:	Gene	Oligo:
26	(SEQ ID NO: 5)	GGATGTTGTTTGTTAGG (SEQ ID NO: 706)
27	(SEQ ID NO: 5)	AGAGCGTCGTTTTGTA (SEQ ID NO: 707)
28	(SEQ ID NO: 5)	AGAGTGTTGTTTTGTAT (SEQ ID NO: 708)
29	(SEQ ID NO: 5)	TTTCGAGGGTAGGCGAG (SEQ ID NO: 709)
30	(SEQ ID NO: 5)	TTTTGAGGGTAGGTGAG (SEQ ID NO: 710)
31	(SEQ ID NO: 5)	TTTCGATTTTAATGCGAA (SEQ ID NO: 711)
32	(SEQ ID NO: 5)	TTTGATTTTAATGTGAAGT (SEQ ID NO: 712)
33	(SEQ ID NO: 5)	AGGAATTTCGTCGCGA (SEQ ID NO: 713)
34	(SEQ ID NO: 5)	AGGAATTTTGTTGTGAT (SEQ ID NO: 714)
35	(SEQ ID NO: 5)	TTTGAGTCGTACGCGT (SEQ ID NO: 715)
36	(SEQ ID NO: 5)	TTTTGAGTTGTATGTGT (SEQ ID NO: 716)
37	(SEQ ID NO: 6)	TACGTAGTTGCGCGTT (SEQ ID NO: 717)
38	(SEQ ID NO: 51)	GTATGTAGTTGTGTGTT (SEQ ID NO: 674)
39	(SEQ ID NO: 6)	AATCGGCGGTTAGGAT (SEQ ID NO: 718)
40	(SEQ ID NO: 6)	GAATTGGTGGTTAGGA (SEQ ID NO: 719)
41	(SEQ ID NO: 6)	TTTGATCGGGTTTGAG (SEQ ID NO: 720)
42	(SEQ ID NO: 6)	TTTTGATTGGGTTTGAG (SEQ ID NO: 721)
43	(SEQ ID NO: 51)	TTTGAGTATTCGTAGGAA (SEQ ID NO: 675)
44	(SEQ ID NO: 6)	TGAGTATTTGTAGGAAGA (SEQ ID NO: 722)
45	(SEQ ID NO: 6)	AGAGGCGCGGGTTATA (SEQ ID NO: 723)
46	(SEQ ID NO: 6)	TAGAGGTGTGGGTTAT (SEQ ID NO: 724)
47	(SEQ ID NO: 6)	TTAGCGGTTAAGTTGCGA (SEQ ID NO: 725)
48	(SEQ ID NO: 6)	TTAGTGGTTAAGTTGTGA (SEQ ID NO: 726)
49	(SEQ ID NO: 8)	TTCGTAGAAGAATACGCGTA (SEQ ID NO: 727)
50	(SEQ ID NO: 8)	TTTGTAGAAGAATATGTGTA (SEQ ID NO: 728)

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No:	Gene	Oligo:
51	(SEQ ID NO: 8)	AAACGTTTATCGGTTG (SEQ ID NO: 729)
52	(SEQ ID NO: 8)	AATGTTTATTGGTTGGA (SEQ ID NO: 730)
53	(SEQ ID NO: 8)	TATCGTAGTTCGTTCGG (SEQ ID NO: 731)
54	(SEQ ID NO: 8)	ATTGTAGTTTGTTTGGT (SEQ ID NO: 732)
55	(SEQ ID NO: 16)	TGGTCGGTATATTTCGA (SEQ ID NO: 733)
56	(SEQ ID NO: 16)	TTGGTTGGTATATTTTGA (SEQ ID NO: 734)
57	(SEQ ID NO: 16)	GGAGGTTTCGGTTCGA (SEQ ID NO: 735)
58	(SEQ ID NO: 16)	TGGAGGTTTTGGTTTGA (SEQ ID NO: 736)
59	(SEQ ID NO: 16)	TTAGCGGTAATAGCGG (SEQ ID NO: 737)
60	(SEQ ID NO: 52)	TATTAGTGGTAATAGTGG (SEQ ID NO: 676)
61	(SEQ ID NO: 42)	TGCGTAGTAGGCGGTT (SEQ ID NO: 738)
62	(SEQ ID NO: 53)	TGTGTAGTAGGTGGTTT (SEQ ID NO: 677)
63	(SEQ ID NO: 42)	TAGGCGGTTGTTCGTA (SEQ ID NO: 739)
64	(SEQ ID NO: 42)	AGGTGGTTGTTTGTAA (SEQ ID NO: 740)
65	(SEQ ID NO: 14)	TTGAAGTCGGTACGGT (SEQ ID NO: 1125)
66	(SEQ ID NO: 14)	TGAAGTTGGTATGGTT (SEQ ID NO: 1126)
67	(SEQ ID NO: 14)	TGGGACGCGGATATTT (SEQ ID NO: 1127)
68	(SEQ ID NO: 14)	GTTGGGATGTGGATAT (SEQ ID NO: 1128)
69	(SEQ ID NO: 43)	GTTCGGGTCGATTCGA (SEQ ID NO: 741)
70	(SEQ ID NO: 43)	GGTTTGGGTTGATTTGA (SEQ ID NO: 742)
71	(SEQ ID NO: 43)	TTCGGGATATATTCGATT (SEQ ID NO: 743)
72	(SEQ ID NO: 43)	TTTTGGGATATATTTGATT (SEQ ID NO: 744)
73	(SEQ ID NO: 43)	TATTCGAATTGTATTCGT (SEQ ID NO: 745)
74	(SEQ ID NO: 43)	TTTGAATTGTATTTGTTTAT (SEQ ID NO: 746)
75	(SEQ ID NO: 15)	TTAAGTTCGATTCGGT (SEQ ID NO: 747)

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No:	Gene	Oligo:
76	(SEQ ID NO: 54)	AGTTTGATTTGGTGAAT (SEQ ID NO: 678)
77	(SEQ ID NO: 15)	TAGTTGTTCGAGAGGG (SEQ ID NO: 748)
78	(SEQ ID NO: 15)	AGTTGTTTGAGAGGGT (SEQ ID NO: 749)
79	(SEQ ID NO: 15)	ATAGTATCGAGGTGAGT (SEQ ID NO: 750)
80	(SEQ ID NO: 15)	ATAGTATTGAGGTGAGTT (SEQ ID NO: 751)
81	(SEQ ID NO: 15)	TTCGGGATTCGATAAT (SEQ ID NO: 752)
82	(SEQ ID NO: 15)	AGGTTTTGGGATTTGA (SEQ ID NO: 753)
83	(SEQ ID NO: 15)	TTAGGACGCGGCGATA (SEQ ID NO: 754)
84	(SEQ ID NO: 15)	AGGATGTGGTGATAGT (SEQ ID NO: 755)
85	(SEQ ID NO: 15)	TTGTACGTTCGGTATT (SEQ ID NO: 756)
86	(SEQ ID NO: 15)	TAAGTTGTATGTTTGGTA (SEQ ID NO: 757)
87	(SEQ ID NO: 4)	GGCGTCGAGGTCGTAG (SEQ ID NO: 758)
88	(SEQ ID NO: 4)	GGTGTTGAGGTTGTAGT (SEQ ID NO: 759)
89	(SEQ ID NO: 4)	AGGGTTTCGATTTTCGG (SEQ ID NO: 760)
90	(SEQ ID NO: 4)	AGGGTTTTGATTTTTGG (SEQ ID NO: 761)
91	(SEQ ID NO: 4)	TGGAACGTGCGATTGT (SEQ ID NO: 762)
92	(SEQ ID NO: 4)	TGGAATGTGTGATTGT (SEQ ID NO: 763)
93	(SEQ ID NO: 4)	TTTTGGCGCGTTTATA (SEQ ID NO: 764)
94	(SEQ ID NO: 4)	TTGGTGTGTTTATAGATA (SEQ ID NO: 765)
95	(SEQ ID NO: 4)	AGATTTTTACGATTCGA (SEQ ID NO: 766)
96	(SEQ ID NO: 4)	TTTTTATGATTTGAAATAGA (SEQ ID NO: 767)
97	(SEQ ID NO: 4)	AGTATTTTCGCGTGTT (SEQ ID NO: 768)
98	(SEQ ID NO: 4)	TAGTATTTTTGTGTGTTAA (SEQ ID NO: 769)
99	(SEQ ID NO: 7)	TTCGTCGGCGGTAGAG (SEQ ID NO: 770)
100	(SEQ ID NO: 7)	TAGAGTTTGTTGGTGG (SEQ ID NO: 771)

No:	Gene	Oligo:
101	(SEQ ID NO: 7)	GATCGCGGGTACGTTT (SEQ ID NO: 772)
102	(SEQ ID NO: 7)	ATTGTGGGTATGTTTGT (SEQ ID NO: 773)
103	(SEQ ID NO: 7)	TTAACGTCGTTGGTTA (SEQ ID NO: 774)
104	(SEQ ID NO: 7)	TGATTAATGTTGTTGGT (SEQ ID NO: 775)
105	(SEQ ID NO: 7)	TTCGCGCGAAGATTTA (SEQ ID NO: 776)
106	(SEQ ID NO: 7)	GTTTTTGTGTGAAGATT (SEQ ID NO: 777)
107	(SEQ ID NO: 44)	TTCGATATCGTGACGG (SEQ ID NO: 778)
108	(SEQ ID NO: 44)	TTTTGATATTGTGATGGT (SEQ ID NO: 779)
109	(SEQ ID NO: 44)	AGAATACGGTCGTAGA (SEQ ID NO: 780)
110	(SEQ ID NO: 44)	TAGAATATGGTTGTAGATA (SEQ ID NO: 781)
111	(SEQ ID NO: 44)	TATTTTTGCGTACGGG (SEQ ID NO: 782)
112	(SEQ ID NO: 44)	ATTTTTGTGTATGGGTT (SEQ ID NO: 783)
113	(SEQ ID NO: 1)	TTACGGTGAAGGCGGA (SEQ ID NO: 784)
114	(SEQ ID NO: 1)	TTATGGTGAAGGTGGA (SEQ ID NO: 785)
115	(SEQ ID NO: 1)	TTCGGGATTAATATCGAGAT (SEQ ID NO: 786)
116	(SEQ ID NO: 1)	TTTGGGATTAATATTGAGAT (SEQ ID NO: 787)
117	(SEQ ID NO: 1)	TTTCGGTTTTCGTTAAT (SEQ ID NO: 788)
118	(SEQ ID NO: 1)	TTTGGTTTTTGTTAATTTAG (SEQ ID NO: 789)
119	(SEQ ID NO: 1)	TGTGCGAAGTTAACGT (SEQ ID NO: 790)
120	(SEQ ID NO: 1)	TTGTGTGAAGTTAATGT (SEQ ID NO: 791)
121	(SEQ ID NO: 1)	AAGTTTATCGGCGTTT (SEQ ID NO: 792)
122	(SEQ ID NO: 1)	AGAAGTTTATTGGTGTTT (SEQ ID NO: 793)
123	(SEQ ID NO: 1)	ATTTCGGAATTTAAGCGT (SEQ ID NO: 794)
124	(SEQ ID NO: 1)	TTTGGAATTTAAGTGTTT (SEQ ID NO: 795)
125	(SEQ ID NO: 1)	TTTTCGCGATTGGAGA (SEQ ID NO: 796)

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No:	Gene	Oligo:
126	(SEQ ID NO: 1)	GTTTTTGTGATTGGAGA (SEQ ID NO: 797)
127	(SEQ ID NO: 1)	ATTTACGCGTTTTAGG (SEQ ID NO: 798)
128	(SEQ ID NO: 1)	ATGGAATTTATGTGTTTT (SEQ ID NO: 799)
129	(SEQ ID NO: 1)	ATGTCGCGGTTTTATA (SEQ ID NO: 800)
130	(SEQ ID NO: 1)	GGATGTTGTGGTTTTAT (SEQ ID NO: 801)
131	(SEQ ID NO: 2)	AGACGGGGTTTACGAG (SEQ ID NO: 802)
132	(SEQ ID NO: 2)	AGATGGGGTTTATGAG (SEQ ID NO: 803)
133	(SEQ ID NO: 2)	TGTCGGTATTAGCGTT (SEQ ID NO: 804)
134	(SEQ ID NO: 2)	GTGTTGGTATTAGTGTT (SEQ ID NO: 805)
135	(SEQ ID NO: 2)	TGGTTTACGTTCGGTA (SEQ ID NO: 806)
136	(SEQ ID NO: 2)	GGTTTATGTTTGGTAGT (SEQ ID NO: 807)
137	(SEQ ID NO: 2)	TTCGTACGGTTAGGTT (SEQ ID NO: 808)
138	(SEQ ID NO: 2)	AGTTTTGTATGGTTAGG (SEQ ID NO: 809)
139	(SEQ ID NO: 45)	ATAGCGATTTCGGCGA (SEQ ID NO: 810)
140	(SEQ ID NO: 45)	AGTGATTTTGGTGAGA (SEQ ID NO: 811)
141	(SEQ ID NO: 45)	GGCGTTTTATTTACGAGA (SEQ ID NO: 812)
142	(SEQ ID NO: 45)	GGGTGTTTTATTTATGAG (SEQ ID NO: 813)
143	(SEQ ID NO: 45)	ATCGTGGACGGTAACGA (SEQ ID NO: 814)
144	(SEQ ID NO: 45)	ATTGTGGATGGTAATGA (SEQ ID NO: 815)
145	(SEQ ID NO: 45)	TTGAGATCGATTCGTT (SEQ ID NO: 816)
146	(SEQ ID NO: 45)	TGAGATTGATTTGTTTAG (SEQ ID NO: 817)
147	(SEQ ID NO: 45)	GGCGAGATTCGTACGT (SEQ ID NO: 818)
148	(SEQ ID NO: 45)	GGTGAGATTTGTATGTT (SEQ ID NO: 819)
149	(SEQ ID NO: 45)	TGACGTTCGTGGTGGA (SEQ ID NO: 820)
150	(SEQ ID NO: 45)	GATGTTTGTGGTGGAG (SEQ ID NO: 821)

No:	Gene	Oligo:
151	(SEQ ID NO: 45)	GTGATCGATTACGGTA (SEQ ID NO: 822)
152	(SEQ ID NO: 45)	AGGTGATTGATTATGGT (SEQ ID NO: 823)
153	(SEQ ID NO: 45)	ATTATTCGTTCGGTGA (SEQ ID NO: 824)
154	(SEQ ID NO: 45)	TATTATTTGTTTGGTGAG (SEQ ID NO: 825)
155	(SEQ ID NO: 45)	TATCGTCGTTAAGTGT (SEQ ID NO: 826)
156	(SEQ ID NO: 45)	TATTATTGTTGTTAAGTGT (SEQ ID NO: 827)
157	(SEQ ID NO: 45)	TGTAAGCGCGAGAATA (SEQ ID NO: 828)
158	(SEQ ID NO: 45)	AGTGTAAGTGTGAGAAT (SEQ ID NO: 829)
159	(SEQ ID NO: 9)	TATAGCGGTTTACGGT (SEQ ID NO: 830)
160	(SEQ ID NO: 9)	TAGTGGTTTATGGTAGT (SEQ ID NO: 831)
161	(SEQ ID NO: 9)	AGGGCGATTAGGACGT (SEQ ID NO: 832)
162	(SEQ ID NO: 9)	AGGGTGATTAGGATGT (SEQ ID NO: 833)
163	(SEQ ID NO: 46)	TTCGTTAGAGTTCGTAG (SEQ ID NO: 834)
164	(SEQ ID NO: 46)	TTTGTTAGAGTTTGTAGT (SEQ ID NO: 835)
165	(SEQ ID NO: 46)	TGAGACGTTTGTCGGT (SEQ ID NO: 836)
166	(SEQ ID NO: 46)	TGAGATGTTTGTTGGT (SEQ ID NO: 837)
167	(SEQ ID NO: 46)	GAAAAGTTCGTCGGTT (SEQ ID NO: 838)
168	(SEQ ID NO: 46)	AGAAAAGTTTGTTGGTT (SEQ ID NO: 839)
169	(SEQ ID NO: 46)	ATGGCGTAGTCGCGAT (SEQ ID NO: 840)
170	(SEQ ID NO: 46)	TGGTGTAGTTGTGATT (SEQ ID NO: 841)
171	(SEQ ID NO: 10)	TTTTGACGTCGATGTA (SEQ ID NO: 842)
172	(SEQ ID NO: 10)	TGATGTTGATGTAGAATT (SEQ ID NO: 843)
173	(SEQ ID NO: 10)	TTGCGATGTGCGTTTA (SEQ ID NO: 844)
174	(SEQ ID NO: 10)	TGTGATGTGTGTTTAGT (SEQ ID NO: 845)
175	(SEQ ID NO: 10)	TGATTACGGCGCGGAT (SEQ ID NO: 846)

No:	Gene	Oligo:
176	(SEQ ID NO: 10)	ATTATGGTGTGGATGG (SEQ ID NO: 847)
177	(SEQ ID NO: 10)	AGATGGCGACGTCGAA (SEQ ID NO: 848)
178	(SEQ ID NO: 10)	ATGGTGATGTTGAAGA (SEQ ID NO: 849)
179	(SEQ ID NO: 10)	TTTAAGCGCGGCGGTA (SEQ ID NO: 850)
180	(SEQ ID NO: 10)	TTTTAAGTGTGGTGGTA (SEQ ID NO: 851)
181	(SEQ ID NO: 10)	AGAAACGTAGACGCGA (SEQ ID NO: 852)
182	(SEQ ID NO: 10)	AATGTAGATGTGATGGA (SEQ ID NO: 853)
183	(SEQ ID NO: 11)	AGAGACGCGAAAAATT (SEQ ID NO: 854)
184	(SEQ ID NO: 11)	TAGAGAGATGTGAAAAAT (SEQ ID NO: 855)
185	(SEQ ID NO: 11)	AGACGAAAGAGTCGTT (SEQ ID NO: 856)
186	(SEQ ID NO: 11)	AGAGATGAAAGAGTTGT (SEQ ID NO: 857)
187	(SEQ ID NO: 11)	TTTTAGTTCGAGCGTA (SEQ ID NO: 858)
188	(SEQ ID NO: 11)	TTAGTTTGAGTGTAGTTA (SEQ ID NO: 859)
189	(SEQ ID NO: 11)	GACGTGAATTTTCGGAA (SEQ ID NO: 860)
190	(SEQ ID NO: 11)	AGGATGTGAATTTTTGG (SEQ ID NO: 861)
191	(SEQ ID NO: 11)	AATGCGTGGTCGTTTT (SEQ ID NO: 862)
192	(SEQ ID NO: 11)	GAAATGTGTGGTTGTT (SEQ ID NO: 863)
193	(SEQ ID NO: 11)	TTTCGTTTGCGGAATT (SEQ ID NO: 864)
194	(SEQ ID NO: 11)	TTTTGTTTGTGGAATTG (SEQ ID NO: 865)
195	(SEQ ID NO: 12)	TGTTCGACGTGATTTT (SEQ ID NO: 866)
196	(SEQ ID NO: 12)	GTGTTTGATGTGATTTT (SEQ ID NO: 867)
197	(SEQ ID NO: 12)	TAACGTTTTTTCGGGT (SEQ ID NO: 868)
198	(SEQ ID NO: 12)	TTAATGTTTTTTTGGGTG (SEQ ID NO: 869)
199	(SEQ ID NO: 12)	TGTTGATTCGGAAATGA (SEQ ID NO: 870)
200	(SEQ ID NO: 12)	TTTGTTGATTTGGAAATG (SEQ ID NO: 871)

No:	Gene	Oligo:
201	(SEQ ID NO: 13)	TAAAGTTTCGAAGCGG (SEQ ID NO: 1129)
202	(SEQ ID NO: 13)	AGTTTTGAAGTGGAGT (SEQ ID NO: 1130)
203	(SEQ ID NO: 13)	AAGTCGGTAGTTATCGT (SEQ ID NO: 1131)
204	(SEQ ID NO: 13)	AAGTTGGTAGTTATTGTT (SEQ ID NO: 1132)
205	(SEQ ID NO: 3)	TTGGAGCGCGAGAAAG (SEQ ID NO: 872)
206	(SEQ ID NO: 3)	TTGGAGTGTGAGAAAG (SEQ ID NO: 873)
207	(SEQ ID NO: 3)	TACGTTATCGGTTCGT (SEQ ID NO: 874)
208	(SEQ ID NO: 3)	TATGTTATTGGTTTGTATT (SEQ ID NO: 875)
209	(SEQ ID NO: 3)	ATTAGGTTCGTGGGCGT (SEQ ID NO: 876)
210	(SEQ ID NO: 3)	ATTAGGTTTGTGGGTGT (SEQ ID NO: 877)
211	(SEQ ID NO: 3)	TGCGGTTTAGAAACGTAG (SEQ ID NO: 878)
212	(SEQ ID NO: 3)	TGTGGTTTAGAAATGTAG (SEQ ID NO: 879)
213	(SEQ ID NO: 3)	GAACGGGTTTCGTAGT (SEQ ID NO: 880)
214	(SEQ ID NO: 3)	GGGAATGGGTTTTGTA (SEQ ID NO: 881)
215	(SEQ ID NO: 3)	TTGCGATAGTCGGCGG (SEQ ID NO: 882)
216	(SEQ ID NO: 3)	TTGTGATAGTTGGTGG (SEQ ID NO: 883)
217	(SEQ ID NO: 3)	AAGAACGGACGTGTTT (SEQ ID NO: 884)
218	(SEQ ID NO: 3)	AGGAAGAATGGATGTG (SEQ ID NO: 885)
219	(SEQ ID NO: 47)	AAGTTTCGTTTGGGAG (SEQ ID NO: 886)
220	(SEQ ID NO: 47)	AAAGTTTTGTTTGGGAG (SEQ ID NO: 887)
221	(SEQ ID NO: 47)	TTGGAAGTCGAAGAGA (SEQ ID NO: 888)
222	(SEQ ID NO: 47)	TTTGGAAGTTGAAGAGA (SEQ ID NO: 889)
223	(SEQ ID NO: 18)	TATCGGGTTCGATTTT (SEQ ID NO: 890)
224	(SEQ ID NO: 18)	GGTGTATTGGGTTTGA (SEQ ID NO: 891)
225	(SEQ ID NO: 20)	TAGGGATTCGCGGAGG (SEQ ID NO: 892)

No:	Gene	Oligo:
226	(SEQ ID NO: 20)	TAGGGATTTGTGGAGG (SEQ ID NO: 893)
227	(SEQ ID NO: 20)	TTGTCGAGTAATTTTCGT (SEQ ID NO: 894)
228	(SEQ ID NO: 20)	TGTTGAGTAATTTTGTT (SEQ ID NO: 895)
229	(SEQ ID NO: 20)	TATTACGGGCGGAGGG (SEQ ID NO: 896)
230	(SEQ ID NO: 20)	TATTATGGGTGGAGGG (SEQ ID NO: 897)
231	(SEQ ID NO: 20)	GACGGTACGTTAGAGG (SEQ ID NO: 898)
232	(SEQ ID NO: 20)	GATGGTATGTTAGAGGT (SEQ ID NO: 899)
233	(SEQ ID NO: 20)	TTGGGCGTCGTTATTA (SEQ ID NO: 900)
234	(SEQ ID NO: 20)	TGGGTGTTGTTATTAGT (SEQ ID NO: 901)
235	(SEQ ID NO: 20)	TATTAGTTCGGTCGTT (SEQ ID NO: 902)
236	(SEQ ID NO: 20)	AGTTTGGTTGTTAGTTT (SEQ ID NO: 903)
237	(SEQ ID NO: 20)	TTATTACGTTTAGCGAT (SEQ ID NO: 904)
238	(SEQ ID NO: 20)	TTTTTATTATGTTTAGTGATA (SEQ ID NO: 905)
239	(SEQ ID NO: 20)	ATAGCGAGTGCGATAT (SEQ ID NO: 906)
240	(SEQ ID NO: 48)	AGGGTCGTAGCGGTAG (SEQ ID NO: 907)
241	(SEQ ID NO: 48)	GAGGGTTGTAGTGGTA (SEQ ID NO: 908)
242	(SEQ ID NO: 50)	TTAGGTCGGACGTAAG (SEQ ID NO: 1143)
243	(SEQ ID NO: 50)	GGTTAGGTTGGATGTA (SEQ ID NO: 1144)
244	(SEQ ID NO: 22)	TAGACGTGGGGTTACGT (SEQ ID NO: 909)
245	(SEQ ID NO: 22)	TAGATGTGGGGTTATGT (SEQ ID NO: 910)
246	(SEQ ID NO: 22)	ATTTCGGGGTAGTATCGT (SEQ ID NO: 911)
247	(SEQ ID NO: 22)	ATTTTGGGGTAGTATTGT (SEQ ID NO: 912)
248	(SEQ ID NO: 19)	TACGCGCGTTTTAAAA (SEQ ID NO: 913)
249	(SEQ ID NO: 19)	TTATGTGTGTTTTAAAATG (SEQ ID NO: 914)
250	(SEQ ID NO: 19)	TACGATATCGTTATATAACGG (SEQ ID NO: 915)

No:	Gene	Oligo:
251	(SEQ ID NO: 19)	TATGATATTGTTATATAATGG (SEQ ID NO: 916)
252	(SEQ ID NO: 19)	TATAGGTTCGCGGTTT (SEQ ID NO: 917)
253	(SEQ ID NO: 19)	TATATAGGTTTGTGGTTT (SEQ ID NO: 918)
254	(SEQ ID NO: 19)	TAGGTGCGCGTTATAT (SEQ ID NO: 919)
255	(SEQ ID NO: 19)	ATGTAGGTGTGTGTTAT (SEQ ID NO: 920)
256	(SEQ ID NO: 55)	TACGTTGTTTGGACGAAT (SEQ ID NO: 679)
257	(SEQ ID NO: 19)	TATGTTGTTTGGATGAAT (SEQ ID NO: 921)
258	(SEQ ID NO: 19)	AAGGAGCGTATTTCGG (SEQ ID NO: 922)
259	(SEQ ID NO: 19)	AGGAGTGTATTTTGGG (SEQ ID NO: 923)
260	(SEQ ID NO: 55)	GTCGGATTTCGGAAGT (SEQ ID NO: 680)
261	(SEQ ID NO: 19)	GTTGGATTTTGGAAGTG (SEQ ID NO: 924)
262	(SEQ ID NO: 19)	GAAGTGACGCGTTCGT (SEQ ID NO: 925)
263	(SEQ ID NO: 19)	GAAGTGATGTGTTTGT (SEQ ID NO: 926)
264	(SEQ ID NO: 19)	TGTTATCGTTGCGCGA (SEQ ID NO: 927)
265	(SEQ ID NO: 19)	ATGTTATTGTTGTGTGA (SEQ ID NO: 928)
266	(SEQ ID NO: 17)	TGAAAACGTTTTTCGT (SEQ ID NO: 929)
267	(SEQ ID NO: 17)	AATGTTTTTTGTAAAGAAA (SEQ ID NO: 930)
268	(SEQ ID NO: 17)	AGGATTTCGGCGTTAT (SEQ ID NO: 931)
269	(SEQ ID NO: 17)	AAAGGATTTTGGTGTTA (SEQ ID NO: 932)
270	(SEQ ID NO: 17)	ATTTATTCGTGCGTTT (SEQ ID NO: 933)
271	(SEQ ID NO: 17)	TATTTGTGTGTTAGGG (SEQ ID NO: 934)
272	(SEQ ID NO: 17)	TTTCGGTGGTTTTCGAA (SEQ ID NO: 935)
273	(SEQ ID NO: 17)	TTTGGTGGTTTTTGAAG (SEQ ID NO: 936)
274	(SEQ ID NO: 17)	GGCGTACGGAATTTTA (SEQ ID NO: 937)
275	(SEQ ID NO: 17)	GGGTGTATGGAATTTTA (SEQ ID NO: 938)

No:	Gene	Oligo:
276	(SEQ ID NO: 17)	TGGACGGAGGTTTCGT (SEQ ID NO: 939)
277	(SEQ ID NO: 17)	TGGATGGAGGTTTTGT (SEQ ID NO: 940)
278	(SEQ ID NO: 17)	TGCGGACGGGATAGTT (SEQ ID NO: 941)
279	(SEQ ID NO: 17)	TGTGGATGGGATAGTT (SEQ ID NO: 942)
280	(SEQ ID NO: 17)	TGATTAGTCGATTCGT (SEQ ID NO: 943)
281	(SEQ ID NO: 17)	GATGTAGGGATGGAGA (SEQ ID NO: 944)
282	(SEQ ID NO: 17)	TATCGTGGTTTTTTACGTAT (SEQ ID NO: 945)
283	(SEQ ID NO: 17)	ATATTGTGGTTTTTTATGTA (SEQ ID NO: 946)
284	(SEQ ID NO: 17)	TTTATTCGGTGTTCGA (SEQ ID NO: 947)
285	(SEQ ID NO: 17)	TATTTGGTGTTTGAGAG (SEQ ID NO: 948)
286	(SEQ ID NO: 23)	GAGGCGCGTTATTTTT (SEQ ID NO: 1133)
287	(SEQ ID NO: 23)	GGGAGGTGTGTTATTTT (SEQ ID NO: 1134)
288	(SEQ ID NO: 23)	AACGGTAGTTAGCGATA (SEQ ID NO: 1135)
289	(SEQ ID NO: 23)	TGAATGGTAGTTAGTGA (SEQ ID NO: 1136)
290	(SEQ ID NO: 23)	TTTTAACGTTCGCGGA (SEQ ID NO: 1137)
291	(SEQ ID NO: 23)	AATGTTTGTGGAGGAT (SEQ ID NO: 1138)
292	(SEQ ID NO: 21)	TTTTTCGCGTATATGTT (SEQ ID NO: 1139)
293	(SEQ ID NO: 21)	TTTTTTGTGTATATGTTTAGG (SEQ ID NO: 1140)
294	(SEQ ID NO: 21)	AAGGGCGGTAAGACGG (SEQ ID NO: 1141)
295	(SEQ ID NO: 21)	AAGGGTGGTAAGATGG (SEQ ID NO: 1142)
296	(SEQ ID NO: 32)	GGTTTCGTTTAATCGT (SEQ ID NO: 949)
297	(SEQ ID NO: 32)	GGGTTTTGTTTAATTGTA (SEQ ID NO: 950)
298	(SEQ ID NO: 32)	GATTCGTATTTCGTAGT (SEQ ID NO: 951)
299	(SEQ ID NO: 32)	TTTGTATTTTGTAGTGGG (SEQ ID NO: 952)
300	(SEQ ID NO: 32)	TTCGTATTTAGCGGAT (SEQ ID NO: 953)

No:	Gene	Oligo:
301	(SEQ ID NO: 32)	GGTTTGTATTTAGTGGA (SEQ ID NO: 954)
302	(SEQ ID NO: 32)	TTAATCGGCGGGTTTT (SEQ ID NO: 955)
303	(SEQ ID NO: 32)	AGTTAATTGGTGGGTT (SEQ ID NO: 956)
304	(SEQ ID NO: 32)	TATTTTGGCGGGTTGTAT (SEQ ID NO: 957)
305	(SEQ ID NO: 32)	TATTTTGGTGGGTTGTAT (SEQ ID NO: 958)
306	(SEQ ID NO: 32)	AAGGTTATCGGTTTAAGA (SEQ ID NO: 959)
307	(SEQ ID NO: 32)	AAGGTTATTGGTTTAAGA (SEQ ID NO: 960)
308	(SEQ ID NO: 32)	GGGGGACGACGTTTTTGT (SEQ ID NO: 961)
309	(SEQ ID NO: 32)	GGGGGATGATGTTTTTGT (SEQ ID NO: 962)
310	(SEQ ID NO: 33)	TTACGGTTCGGTTATT (SEQ ID NO: 963)
311	(SEQ ID NO: 33)	AGGTTTTATGGTTTGGT (SEQ ID NO: 964)
312	(SEQ ID NO: 33)	GACGTCGCGGGGTTAG (SEQ ID NO: 965)
313	(SEQ ID NO: 33)	TGATGTTGTGGGGTTA (SEQ ID NO: 966)
314	(SEQ ID NO: 33)	AGGTATTTCGCGATAT (SEQ ID NO: 967)
315	(SEQ ID NO: 33)	AGGTATTTTGTGATATTT (SEQ ID NO: 968)
316	(SEQ ID NO: 33)	GTTTTTCGATTTACGTT (SEQ ID NO: 969)
317	(SEQ ID NO: 33)	TAGGTTTTTTGATTTATGT (SEQ ID NO: 970)
318	(SEQ ID NO: 33)	GGTAGTTTCGATTATTTA (SEQ ID NO: 971)
319	(SEQ ID NO: 33)	GGTAGTTTTGATTATTTA (SEQ ID NO: 972)
320	(SEQ ID NO: 33)	TAGAGTACGGGGCGGG (SEQ ID NO: 973)
321	(SEQ ID NO: 33)	TAGAGTATGGGGTGGG (SEQ ID NO: 974)
322	(SEQ ID NO: 33)	TTGTTTAGCGGATTTTAG (SEQ ID NO: 975)
323	(SEQ ID NO: 33)	TTGTTTAGTGGATTTTAG (SEQ ID NO: 976)
324	(SEQ ID NO: 33)	TAGGTTCGGTTCGTTAT (SEQ ID NO: 977)
325	(SEQ ID NO: 33)	TAGGTTTGGTTTGTTATT (SEQ ID NO: 978)

No:	Gene	Oligo:
326	(SEQ ID NO: 33)	TGGTGGTACGTAGTTTGG (SEQ ID NO: 979)
327	(SEQ ID NO: 33)	TTTGGCGTAGATCGGT (SEQ ID NO: 980)
328	(SEQ ID NO: 33)	TTTGGTGTAGATTGGT (SEQ ID NO: 981)
329	(SEQ ID NO: 33)	AGTGTTCGTCGTAGTT (SEQ ID NO: 982)
330	(SEQ ID NO: 33)	TGAGTGTTTGTTGTAGT (SEQ ID NO: 983)
331	(SEQ ID NO: 33)	GTGTTTAGCGCGGATT (SEQ ID NO: 984)
332	(SEQ ID NO: 33)	GGTGTTTAGTGTGGATT (SEQ ID NO: 985)
333	(SEQ ID NO: 34)	TTCGGTTAGTTTCGTAT (SEQ ID NO: 986)
334	(SEQ ID NO: 34)	TTTTGGTTAGTTTTGTATT (SEQ ID NO: 987)
335	(SEQ ID NO: 34)	GATTCGTTTGGGTAACGT (SEQ ID NO: 988)
336	(SEQ ID NO: 34)	GATTTGTTTGGGTAATGT (SEQ ID NO: 989)
337	(SEQ ID NO: 34)	GTCGAATTTAGTCGCGT (SEQ ID NO: 990)
338	(SEQ ID NO: 34)	GTTGAATTTAGTTGTGTA (SEQ ID NO: 991)
339	(SEQ ID NO: 34)	AATTCGCGAGTTTAGA (SEQ ID NO: 992)
340	(SEQ ID NO: 34)	AAAAATTTGTGAGTTTAG (SEQ ID NO: 993)
341	(SEQ ID NO: 24)	AGGGGTTCGATTAGGG (SEQ ID NO: 1145)
342	(SEQ ID NO: 24)	AGGGGTTTGATTAGGG (SEQ ID NO: 1146)
343	(SEQ ID NO: 24)	TTAGGTATACGAAAGAGTAT (SEQ ID NO: 1147)
344	(SEQ ID NO: 24)	TTAGGTATATGAAAGAGTAT (SEQ ID NO: 1148)
345	(SEQ ID NO: 24)	TGTCGTACGTTATGTT (SEQ ID NO: 1149)
346	(SEQ ID NO: 24)	GGTGTTGTATGTTATGT (SEQ ID NO: 1150)
347	(SEQ ID NO: 24)	TTGATTGGCGGACGAG (SEQ ID NO: 1151)
348	(SEQ ID NO: 24)	TTGATTGGTGGATGAG (SEQ ID NO: 1152)
349	(SEQ ID NO: 35)	TATATATACGTGTGGGTA (SEQ ID NO: 994)
350	(SEQ ID NO: 35)	TATATATATGTGTGGGTA (SEQ ID NO: 995)

No:	Gene	Oligo:
351	(SEQ ID NO: 35)	TATGTAGTCGCGTAGT (SEQ ID NO: 996)
352	(SEQ ID NO: 35)	TTTATGTAGTTGTGTAGT (SEQ ID NO: 997)
353	(SEQ ID NO: 35)	AGTGTATGCGTAGAAGGT (SEQ ID NO: 998)
354	(SEQ ID NO: 35)	AGTGTATGTGTAGAAGGT (SEQ ID NO: 999)
355	(SEQ ID NO: 35)	TTTAGATACGAAATGTTA (SEQ ID NO: 1000)
356	(SEQ ID NO: 35)	TTTAGATATGAAATGTTA (SEQ ID NO: 1001)
357	(SEQ ID NO: 35)	AAGTAAGTCGTTGTTGTT (SEQ ID NO: 1002)
358	(SEQ ID NO: 35)	AAGTAAGTTGTTGTTGTT (SEQ ID NO: 1003)
359	(SEQ ID NO: 25)	TTTCGTCGGAGGAATT (SEQ ID NO: 1004)
360	(SEQ ID NO: 25)	GTTTGTTGGAGGAATT (SEQ ID NO: 1005)
361	(SEQ ID NO: 25)	ATCGTTTTGTCGGACGG (SEQ ID NO: 1006)
362	(SEQ ID NO: 25)	ATTGTTTTGTTGGATGG (SEQ ID NO: 1007)
363	(SEQ ID NO: 25)	TGTCGCGATATATCGA (SEQ ID NO: 1008)
364	(SEQ ID NO: 25)	TTTGTTGTGATATATTGAT (SEQ ID NO: 1009)
365	(SEQ ID NO: 36)	AGCGTCGATTAATCGT (SEQ ID NO: 1010)
366	(SEQ ID NO: 36)	TTAAGTGTTGATTAATTGT (SEQ ID NO: 1011)
367	(SEQ ID NO: 36)	TTCGGTCGGGTTTAAA (SEQ ID NO: 1012)
368	(SEQ ID NO: 36)	GTTTGGTTGGGTTTAAA (SEQ ID NO: 1013)
369	(SEQ ID NO: 36)	TAATCGTTAGCGGCGG (SEQ ID NO: 1014)
370	(SEQ ID NO: 36)	TTAATTGTTAGTGGTGG (SEQ ID NO: 1015)
371	(SEQ ID NO: 36)	TTAACGGGTGGGTACGT (SEQ ID NO: 1016)
372	(SEQ ID NO: 36)	TTAATGGGTGGGTATGT (SEQ ID NO: 1017)
373	(SEQ ID NO: 36)	AGGTCGTTGGTATTCGT (SEQ ID NO: 1018)
374	(SEQ ID NO: 36)	AGGTTGTTGGTATTTGT (SEQ ID NO: 1019)
375	(SEQ ID NO: 36)	TTTTCGAGTTTTATCGA (SEQ ID NO: 1020)

No:	Gene	Oligo:
376	(SEQ ID NO: 36)	TTTGAGTTTTATTGAGGT (SEQ ID NO: 1021)
377	(SEQ ID NO: 36)	ATAGTCGTGGTTTCGT (SEQ ID NO: 1022)
378	(SEQ ID NO: 36)	ATAGTTGTGGTTTTGTT (SEQ ID NO: 1023)
379	(SEQ ID NO: 36)	TGACGGGCGTTTTCGA (SEQ ID NO: 1024)
380	(SEQ ID NO: 36)	GATGGGTGTTTTTGAG (SEQ ID NO: 1025)
381	(SEQ ID NO: 36)	TAATGAGCGCGTTGTA (SEQ ID NO: 1026)
382	(SEQ ID NO: 36)	ATGAGTGTGTTGTATTT (SEQ ID NO: 1027)
383	(SEQ ID NO: 28)	TTGGTTCGGGAAAGGTAA (SEQ ID NO: 1028)
384	(SEQ ID NO: 28)	TTGGTTTGGGAAAGGTAA (SEQ ID NO: 1029)
385	(SEQ ID NO: 28)	TTTCGGTGAATCGGAT (SEQ ID NO: 1030)
386	(SEQ ID NO: 28)	TTTTTGGTGAATTGGAT (SEQ ID NO: 1031)
387	(SEQ ID NO: 28)	TTCGTAAAGTCGTTGT (SEQ ID NO: 1032)
388	(SEQ ID NO: 28)	GGTTTTTTGTAAAGTTGT (SEQ ID NO: 1033)
389	(SEQ ID NO: 28)	GTTTAGTTAGCGGGTTTT (SEQ ID NO: 1034)
390	(SEQ ID NO: 28)	GTTTAGTTAGTGGGTTTT (SEQ ID NO: 1035)
391	(SEQ ID NO: 28)	GGGCGCGTACGGTTAT (SEQ ID NO: 1036)
392	(SEQ ID NO: 28)	AGTTGGGTGTGTATGG (SEQ ID NO: 1037)
393	(SEQ ID NO: 28)	TTATCGCGCGTGGAGG (SEQ ID NO: 1038)
394	(SEQ ID NO: 28)	TTATTGTGTGTGGAGGA (SEQ ID NO: 1039)
395	(SEQ ID NO: 37)	AAAACGTGGACGTTTT (SEQ ID NO: 1153)
396	(SEQ ID NO: 37)	ATTTGGAGCGAGGAATTT (SEQ ID NO: 1154)
397	(SEQ ID NO: 37)	ATTTGGAGTGAGGAATTT (SEQ ID NO: 1155)
398	(SEQ ID NO: 37)	TTGAAAGTCGGTTAAAGT (SEQ ID NO: 1156)
399	(SEQ ID NO: 37)	TTGAAAGTTGGTTAAAGT (SEQ ID NO: 1157)
400	(SEQ ID NO: 37)	GGTAGTTACGAGAGAGTT (SEQ ID NO: 1158)

No:	Gene	Oligo:
401	(SEQ ID NO: 37)	GGTAGTTATGAGAGAGTT (SEQ ID NO: 1159)
402	(SEQ ID NO: 26)	GGTGCGCGTAGAGAAT (SEQ ID NO: 1040)
403	(SEQ ID NO: 26)	GGTGTGTGTAGAGAATA (SEQ ID NO: 1041)
404	(SEQ ID NO: 26)	TAAGCGTATCGACGTT (SEQ ID NO: 1042)
405	(SEQ ID NO: 26)	ATTTTAAGTGTATTGATGT (SEQ ID NO: 1043)
406	(SEQ ID NO: 26)	AAATATCGAACGGGAT (SEQ ID NO: 1044)
407	(SEQ ID NO: 26)	ATTGAATGGGATTTAGAG (SEQ ID NO: 1045)
408	(SEQ ID NO: 26)	TTAGAGTTCGAGTTTATA (SEQ ID NO: 1046)
409	(SEQ ID NO: 26)	TTAGAGTTTGAGTTTATA (SEQ ID NO: 1047)
410	(SEQ ID NO: 26)	TTAGGCGCGGATTCGT (SEQ ID NO: 1048)
411	(SEQ ID NO: 26)	TAGGTGTGGATTTGTT (SEQ ID NO: 1049)
412	(SEQ ID NO: 26)	TTCGCGAAGTTACGGG (SEQ ID NO: 1050)
413	(SEQ ID NO: 26)	TTTGTGAAGTTATGGGT (SEQ ID NO: 1051)
414	(SEQ ID NO: 26)	TATCGGTTCGGAGTTA (SEQ ID NO: 1052)
415	(SEQ ID NO: 26)	ATTGGTTTGGAGTTAGA (SEQ ID NO: 1053)
416	(SEQ ID NO: 26)	AAGTAGCGTCGTTATT (SEQ ID NO: 1054)
417	(SEQ ID NO: 26)	AAGTAGTGTTGTTATTGA (SEQ ID NO: 1055)
418	(SEQ ID NO: 26)	GTCGTTCGGAATTCGT (SEQ ID NO: 1056)
419	(SEQ ID NO: 26)	AGTTGTTTGGAATTTGT (SEQ ID NO: 1057)
420	(SEQ ID NO: 26)	TACGTGGTCGAGGGTT (SEQ ID NO: 1058)
421	(SEQ ID NO: 26)	TATGTGGTTGAGGGTT (SEQ ID NO: 1059)
422	(SEQ ID NO: 26)	GGAAGTTTCGATGGTTTA (SEQ ID NO: 1060)
423	(SEQ ID NO: 26)	GGAAGTTTTGATGGTTTA (SEQ ID NO: 1061)
424	(SEQ ID NO: 38)	GGCGTTGGTATCGTTGA (SEQ ID NO: 1062)
425	(SEQ ID NO: 38)	GGTGTTGGTATTGTTGA (SEQ ID NO: 1063)

No:	Gene	Oligo:
426	(SEQ ID NO: 38)	TTAAGACGCGTTTTTT (SEQ ID NO: 1064)
427	(SEQ ID NO: 38)	AAGATGTGTTTTTGGA (SEQ ID NO: 1065)
428	(SEQ ID NO: 38)	TTTTGTCGCGGGAATT (SEQ ID NO: 1066)
429	(SEQ ID NO: 38)	TTTTTGTTGTGGGAATT (SEQ ID NO: 1067)
430	(SEQ ID NO: 38)	ATACGTAGATTCGGAG (SEQ ID NO: 1068)
431	(SEQ ID NO: 38)	TATGTAGATTTGGAGGT (SEQ ID NO: 1069)
432	(SEQ ID NO: 39)	GAAGTGGTCGTTAGTTTT (SEQ ID NO: 1070)
433	(SEQ ID NO: 39)	GAAGTGGTTGTTAGTTTTT (SEQ ID NO: 1071)
434	(SEQ ID NO: 39)	AAGGAATTCGTTTTGTAA (SEQ ID NO: 1072)
435	(SEQ ID NO: 39)	AAGGAATTTGTTTTGTAA (SEQ ID NO: 1073)
436	(SEQ ID NO: 39)	AATGTTTTCGTGATGTTG (SEQ ID NO: 1074)
437	(SEQ ID NO: 39)	AATGTTTTTGTGATGTTG (SEQ ID NO: 1075)
438	(SEQ ID NO: 39)	TAAAACGAGGGAGCGT (SEQ ID NO: 1076)
439	(SEQ ID NO: 39)	AAAATGAGGGAGTGTT (SEQ ID NO: 1077)
440	(SEQ ID NO: 27)	AGGAGTCGGTTTCGTA (SEQ ID NO: 1078)
441	(SEQ ID NO: 27)	AGGAGTTGGTTTTGTA (SEQ ID NO: 1079)
442	(SEQ ID NO: 27)	TAAAGCGCGGATATTT (SEQ ID NO: 1080)
443	(SEQ ID NO: 27)	GGGTAAAGTGTGGATA (SEQ ID NO: 1081)
444	(SEQ ID NO: 27)	TTTGAGCGGGTATCGA (SEQ ID NO: 1082)
445	(SEQ ID NO: 27)	TGAGTGGGTATTGAGT (SEQ ID NO: 1083)
446	(SEQ ID NO: 27)	TAGAGTCGAGGGGCGG (SEQ ID NO: 1084)
447	(SEQ ID NO: 27)	TAGAGTTGAGGGGTGG (SEQ ID NO: 1085)
448	(SEQ ID NO: 27)	TTTCGAGGGACGGAAG (SEQ ID NO: 1086)
449	(SEQ ID NO: 27)	TTTTGAGGGATGGAAG (SEQ ID NO: 1087)
450	(SEQ ID NO: 27)	TATGTTTTCGGCGAAT (SEQ ID NO: 1088)

No:	Gene	Oligo:
451	(SEQ ID NO: 27)	TTTTGGTGAATGGGGA (SEQ ID NO: 1089)
452	(SEQ ID NO: 27)	ATAGTCGAGGAGTCGT (SEQ ID NO: 1090)
453	(SEQ ID NO: 27)	AATAGTTGAGGAGTTGT (SEQ ID NO: 1091)
454	(SEQ ID NO: 29)	ATTTGTTTCGATTAATTT (SEQ ID NO: 1092)
455	(SEQ ID NO: 29)	ATTTGTTTTGATTAATTT (SEQ ID NO: 1093)
456	(SEQ ID NO: 29)	AATTTGCGAACGTTTGGG (SEQ ID NO: 1094)
457	(SEQ ID NO: 29)	AATTTGTGAATGTTTGGG (SEQ ID NO: 1095)
458	(SEQ ID NO: 29)	GTCGATGTTTTCGGTA (SEQ ID NO: 1096)
459	(SEQ ID NO: 29)	GGTTGATGTTTTGGTA (SEQ ID NO: 1097)
460	(SEQ ID NO: 31)	GAGTTTCGTTATATCGT (SEQ ID NO: 1098)
461	(SEQ ID NO: 31)	GGAGTTTTGTTATATTGT (SEQ ID NO: 1099)
462	(SEQ ID NO: 31)	TTTTTGCGGTTCGATA (SEQ ID NO: 1100)
463	(SEQ ID NO: 31)	AATTTTTGTGGTTTGATA (SEQ ID NO: 1101)
464	(SEQ ID NO: 31)	TACGTTAAGGTTAACGTATA (SEQ ID NO: 1102)
465	(SEQ ID NO: 31)	TATGTTAAGGTTAATGTATA (SEQ ID NO: 1103)
466	(SEQ ID NO: 31)	TGTTTCGTCGTTATAAT (SEQ ID NO: 1104)
467	(SEQ ID NO: 31)	GTTTTGTTGTTATAATTAGA (SEQ ID NO: 1105)
468	(SEQ ID NO: 31)	GGCGTAGGTTACGATT (SEQ ID NO: 1106)
469	(SEQ ID NO: 31)	GGGGTGTAGGTTATGA (SEQ ID NO: 1107)
470	(SEQ ID NO: 31)	ATTCGTTACGGATCGT (SEQ ID NO: 1108)
471	(SEQ ID NO: 31)	TTTATTTGTTATGGATTGT (SEQ ID NO: 1109)
472	(SEQ ID NO: 31)	AGTTTTCGGATTCGAA (SEQ ID NO: 1110)
473	(SEQ ID NO: 31)	AGAGTTTTTGGATTTGA (SEQ ID NO: 1111)
474	(SEQ ID NO: 31)	TATTTCGAGGTAGCGG (SEQ ID NO: 1112)
475	(SEQ ID NO: 31)	TTTGAGGTAGTGGGAT (SEQ ID NO: 1113)

No:	Gene	Oligo:
476	(SEQ ID NO: 31)	GAGAGAAACGGTTTTTGT (SEQ ID NO: 1114)
477	(SEQ ID NO: 31)	GAGAGAAATGGTTTTTGT (SEQ ID NO: 1115)
478	(SEQ ID NO: 31)	GTTTGATGGATGTTTTT (SEQ ID NO: 1116)
479	(SEQ ID NO: 31)	GTACGACGGTAAGGAT (SEQ ID NO: 1117)
480	(SEQ ID NO: 31)	GTATGATGGTAAGGATTA (SEQ ID NO: 1118)
481	(SEQ ID NO: 31)	AGTTGTTTCGTAGATATT (SEQ ID NO: 1119)
482	(SEQ ID NO: 31)	AGTTGTTTTGTAGATATT (SEQ ID NO: 1120)
483	(SEQ ID NO: 40)	AGTAAGCGGTTGTATAT (SEQ ID NO: 1121)
484	(SEQ ID NO: 40)	AAAAGTAAGTGGTTGTAT (SEQ ID NO: 1122)
485	(SEQ ID NO: 40)	AAATTGAGCGTTTATGT (SEQ ID NO: 1123)
486	(SEQ ID NO: 40)	ATTGAGTGTTTATGTGTA (SEQ ID NO: 1124)

Table 3: Genes or genomic locus associated with genomic sequences according to SEQ ID NO:1- SEQ ID NO:58.

Genomic SEQ ID NO:	Bisulfite SEQ ID Nos:	Genbank Ref. ID No:/contig	Description
1	304, 305, 420 & 421	AL355481.12.1.12 2435	Hypothetical protein-leucine rich repeat (Vega gene ID OTTHUMG00013001328)
2	306. 307, 422 & 423	AC027601.25.1.19 2732	5 azacytidine induced (Ensembl gene ID 141577)
3	308, 309, 424 & 425	AL831711.4.1.880 1	No known gene, close to ATP/GTP-binding site motif A (P-loop) protein
4	310, 311, 426 & 427	NM_002938	RING FINGER PROTEIN 4; RNF4
5	312, 313, 428 & 429	NM_021926	HOMEOBOX PROTEIN ARISTALESS-LIKE 4;. ALX 4
6	314, 315, 430 & 431	NM_004852	ONE CUT DOMAIN FAMILY MEMBER 2; ONC2

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Genomic SEQ ID NO:	Bisulfite SEQ ID Nos:	Genbank Ref. ID No:/contig	Description
7	316, 317, 432 & 433	NM_002507	TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY MEMBER 16 PRECURSOR (LOW- AFFINITY NERVE GROWTH FACTOR RECEPTOR) (NGF RECEPTOR) (GP80-LNGFR) (P75 ICD) (LOW AFFINITY NEUROTROPHIN RECEPTOR P75NTR).
8	318, 319, 434 & 435	NM_016192	TRANSMEMBRANE PROTEIN WITH EGF-LIKE AND TWO FOLLISTATIN-LIKE DOMAINS 2; TRANSMEMBRANE PROTEIN TENB2; TOMOREGULIN; PUTATIVE TRANSMEMBRANE PROTEIN WITH EGF-LIKE AND TWO FOLLISTATIN-LIKE DOMAINS 2.
9	320, 321, 436 & 437	NM_005221	HOMEOBOX PROTEIN DLX-5
10	322, 323, 438 & 439	NM_080552	VESICULAR INHIBITORY AMINO ACID TRANSPORTER (GABA AND GLYCINE TRANSPORTER) (VESICULAR GABA TRANSPORTER) (HVIAAT)
11	324, 325, 440 & 441	NM_001208	Transcription factor BTF3 Homolog 1
12	326, 327, 442 & 443	NM_005904	MOTHERS AGAINST DECAPENTAPLEGIC HOMOLOG 7 (SMAD 7) (MOTHERS AGAINST DPP HOMOLOG 7) (SMAD7) (HSMAD7).
13	328, 329, 444 & 445	NM_002146	HOMEOBOX PROTEIN HOX-B3 (HOX-2G) (HOX-2.7)
14	330, 331, 446 & 447	NM_025078	Homo sapiens hypothetical protein FLJ22378
15	332, 333, 448 & 449	NM_001116	ADENYLATE CYCLASE, TYPE IX (EC 4.6.1.1) (ATP PYROPHOSPHATE-LYASE) (ADENYLYL CYCLASE).
16	334, 335, 450 & 451	NM_001706	B-CELL LYMPHOMA 6 PROTEIN (BCL-6) (ZINC FINGER PROTEIN 51) (LAZ-3 PROTEIN) (BCL-5).
17	336, 337, 452 & 453	NM_014068	SEEK1 PROTEIN
18	338, 339, 454 & 455	NM_017745	BCL-6 INTERACTING COREPRESSOR ISOFORM 1
19	340, 341, 456 & 457	NM_005643	TRANSCRIPTION INITIATION FACTOR TFIID 28 KDA SUBUNIT (TAFII-28) (TAFII28) (TFIID SUBUNIT P30-BETA)

Genomic SEQ ID NO:	Bisulfite SEQ ID Nos:	Genbank Ref. ID No:/contig	Description
20	342, 343, 458 & 459	NM_007374	HOMEOBOX PROTEIN SIX6 (SINE OCULIS HOMEOBOX HOMOLOG 6) (OPTIC HOMEOBOX 2) (HOMEODOMAIN PROTEIN OPTX2).
21	344, 345, 460 & 461	AP003500.2.1.161 078	Situated between Ensemble gene IDs ENSESTG00002308609 and ENSESTG00002308691
22	346, 347, 462 & 463	AC092385.4.1.972 18	SPIR-2 PROTEIN (FRAGMENT)
23	348, 349, 464 & 465	AC007223.5.1.159 520	Situated within Ensemble EST ID ENSESTG00001971415
24	350, 351, 466 & 467	NM_005229	ETS-DOMAIN PROTEIN ELK-1
25	352, 353, 468 & 469	NM_000179	DNA MISMATCH REPAIR PROTEIN MSH6 (MUTS-ALPHA 160 KDA SUBUNIT) (G/T MISMATCH BINDING PROTEIN) (GTBP) (GTMBP) (P160)
26	354, 355, 470 & 471	NM_005427	TUMOR PROTEIN P73 (P53-LIKE TRANSCRIPTION FACTOR) (P53-RELATED PROTEIN).
27	356, 357, 472 & 473	NM_002093	GLYCOGEN SYNTHASE KINASE-3 BETA (EC 2.7.1.37) (GSK-3 BETA).
28	358, 359, 474 & 475	NM_006765	N33 PROTEIN
29	360, 361, 476 & 477	NM_016192	TRANSMEMBRANE PROTEIN WITH EGF-LIKE AND TWO FOLLISTATIN-LIKE DOMAINS 2; TRANSMEMBRANE PROTEIN TENB2; TOMOREGULIN; PUTATIVE TRANSMEMBRANE PROTEIN WITH EGF-LIKE AND TWO FOLLISTATIN-LIKE DOMAINS 2.
30	362, 363, 478 & 479	NM_004429	EPHRIN-B1 PRECURSOR (EPH-RELATED RECEPTOR TYROSINE KINASE LIGAND 2) (LERK-2) (ELK LIGAND) (ELK-L).
31	364, 365, 480 & 481	NM_018950	HLA-F gene for human leukocyte antigen F
32	366, 367, 482 & 483	NM_000038	ADENOMATOUS POLYPOSIS COLI PROTEIN (APC PROTEIN)

Genomic SEQ ID NO:	Bisulfite SEQ ID Nos:	Genbank Ref. ID No:/contig	Description
33	368, 369, 484 & 485	NM_000610	CD44 ANTIGEN PRECURSOR (PHAGOCYTIC GLYCOPROTEIN I) (PGP-1) (HUTCH-I) (EXTRACELLULAR MATRIX RECEPTOR-III) (ECMR-III) (GP90 LYMPHOCYTE HOMING/ADHESION RECEPTOR) (HERMES ANTIGEN) (HYALURONATE RECEPTOR) (HEPARAN SULFATE PROTEOGLYCAN) (EPICAN) (CDW44)
34	370, 371, 486 & 487	NM_004385	VERSICAN CORE PROTEIN PRECURSOR (LARGE FIBROBLAST PROTEOGLYCAN) (CHONDROITIN SULFATE PROTEOGLYCAN CORE PROTEIN 2) (PG-M) (GLIAL HYALURONATE-BINDING PROTEIN) (GHAP)
35	372, 373, 488 & 489		EYA 4 EnsEMBL ID <u>ENSG00000112319</u>
36	374, 375, 490 & 491	NM_000455	SERINE/THREONINE-PROTEIN KINASE 11 (EC 2.7.1.-) (SERINE/THREONINE- PROTEIN KINASE LKB1)
37	376, 377, 492 & 493	NM_003242	Transforming growth factor, beta receptor II (TGFBR2)
38	378, 379, 494 & 495	NM_001753	CAVEOLIN-1; CAV 1
39	380, 381, 496 & 497	NM_001257	CADHERIN-13 PRECURSOR (TRUNCATED-CADHERIN) (T-CADHERIN) (T-CAD) (HEART-CADHERIN) (H-CADHERIN) (P105)
40	382, 383, 498 & 499	NM_000044	ANDROGEN RECEPTOR (DIHYDROTESTOSTERONE RECEPTOR)
41	384, 385, 500 & 501	NM_032546	RING FINGER PROTEIN 30
42	386, 387, 502 & 503	NM_033178	DOUBLE HOMEOBOX, 4; DOUBLE HOMEOBOX PROTEIN 4.
43	388, 389, 504 & 505	NM_003573	LATENT TRANSFORMING GROWTH FACTOR BETA BINDING PROTEIN 4.; LTBP4
44	390, 391, 506 & 507		CGI-20 PROTEIN
45	392, 393, 508 & 509	NM_014459	PROTOCADHERIN 17; PROTOCADHERIN 68.; PCDH 17
46	394, 395, 510 & 511	NM_005285	PROBABLE G PROTEIN-COUPLED RECEPTOR GPR7.; GPR7

Genomic SEQ ID NO:	Bisulfite SEQ ID Nos:	Genbank Ref. ID No:/contig	Description
47	396, 397, 512 & 513	NM_016269	LYMPHOID ENHANCER BINDING FACTOR 1 (LEF-1) (T CELL-SPECIFIC TRANSCRIPTION FACTOR 1-ALPHA) (TCF1-ALPHA)
48	398, 399, 514 & 515	AC139426.2.1.188448	Situated between EnsEMBL ID ENSESTG00003302072 and EnsEMBL ID ENSESTG00003302068
49	400, 401, 516 & 517	NM_005904	MOTHERS AGAINST DECAPENTAPLEGIC HOMOLOG 7 (SMAD 7) (MOTHERS AGAINST DPP HOMOLOG 7) (SMAD7) (HSMAD7).
50	402, 403, 518 & 519	AL512590.2.1.165432	Homo sapiens mRNA for KIAA1529 protein EnsEMBL ID ENSG00000029402
51	404, 405, 520 & 521	NM_004852	ONE CUT DOMAIN FAMILY MEMBER 2 (ONECUT-2 TRANSCRIPTION FACTOR) (OC-2).
52	406, 407, 522 & 523	NM_001900	CYSTATIN D PRECURSOR
53	408, 409, 524 & 525	NM_001990	EYES ABSENT HOMOLOG 3. EYA3
54	410, 411, 526 & 527	NM_002032	FERRITIN HEAVY CHAIN (FERRITIN H SUBUNIT).
55	412, 413, 528 & 529	NM_002938	RING FINGER PROTEIN 4.; RNF4
56	414, 415, 530 & 531		HPP1 EnsEMBL ID ENSG00000144339
57	416, 417, 532 & 533	NM_000852	GLUTATHIONE S-TRANSFERASE P (EC 2.5.1.18) (GST CLASS-PI) (GSTP1-1)
58	418, 419, 534 & 535	NM_007084	TRANSCRIPTION FACTOR SOX-21